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THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

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THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

The accounts of the Marine Biological Laboratory for the year 1947 have been audited as heretofore by Messrs. Seamans, Stetson and Tuttle, certified public accountants of Boston, and a copy of their audit is available for inspection at any time in the Laboratory office.

There were few changes in the Balance Sheet from the preceding year, and it is appended as Exhibit A. As of December 31, 1947, the total *book* value of all the Endowment Assets, including the Scholarship Funds, was $978,677.67, an increase of $1,478.37. The securities and cash comprising these assets had at the end of the year a *market* value of $989,814.16. Plant Assets (Land, Buildings and Equipment) amounted to $1,311,950.55, a reduction for the year of $1,013.55. Current Assets were increased $10,156.31 to a total of $231,008.19. Current Liabilities (Accounts Payable) were $11,621.59.

In recent years the Treasurer's Report has also included the "Exhibit B—Income and Expense" of the Auditors' Report. This year there is being substituted a summary of "Receipts and Expenditures" prepared by the Treasurer giving the actual cash transactions during the year. The Auditors' statement of "Income and Expense" is a necessary accounting of the books of the Laboratory as they are set up, but does not, in the Treasurer's judgment, give a simple picture of the moneys received and spent during the year. It includes items of a purely bookkeeping character such as depreciation, the value of Library serials received through exchange, and interdepartmental charges. It does not include expenditures for capital items including some apparatus, books and serials for the Library, etc., even though some of these are normal operating expenses, nor either pensions paid nor funds paid into the Retirement Fund. (All of these are accounted for elsewhere in the Auditors' Report, Schedule IV or Exhibit B.) It does include all gifts in "income" even though some of these gifts are designated for such special purposes as a new boat, and cannot be used for current expenses.

Statement I which follows is a summary of the actual financial transactions of the year except for donations for special purposes, special agency accounts, and real estate development accounts which are listed subsequently. Actual receipts in 1947 for current operations were $250,098.72. Current expenditures were $253,822.09 and are listed in detail by departments in "II. Current Expenses." An additional $32,806.56 was spent on repairs and special purchases entered on the books as "Plant Assets" and listed below in "III. Additions to Capital Assets from Current Funds." The total of the year's expenditures is therefore $286,628.65. After the deduction of $2,671.07 transferred from the Carnegie Book Fund and used for the purchase of some of the Library items, the resulting cash deficit for the year is $33,858.86. This was taken care of by using $10,000 of the Reserve Fund (reducing it to $6,218.88) and by reducing the cash balances in the checking accounts to a total of $7,949.16 at the end of the year.

It should be noted that at the end of the year there was a net increase of the excess of Accounts Receivable over Accounts Payable amounting to $5,776.01, and an increase in the value of Supply Department inventory of $4,848.29. If these two items are taken into account, the actual deficit for the year is reduced to $23,234.56. On the other hand, if the reserve for depreciation is deducted, as it properly is in the Auditors' Report, the deficit would be $25,806.10 greater or $49,040.66.

1. *Cash Statement for Year Ended December 31, 1947*

		Expenditures		
	Receipts	Current	Additions to Capital Assets	Total Expenditures
Membership Dues	$ 2,238.00			
Donations for Current Expenses [1]	944.95			
Income from Endowment	35,616.77			
Income other Securities	21,492.00			
Real Estate Rentals	6,360.00	$ 1,002.84		$ 1,002.84
Instruction	12,630.00	7,824.94		7,824.94
Research (incl. Apparatus and Chemical Depts.)	25,193.34	17,372.60	$ 2,127.22	19,499.82
Mess	32,029.42	33,965.04	1,880.93	35,845.87
Dormitories and Apt. House	16,600.10	13,570.83	11,013.25	24,584.08
Library [2]	3,000.00	11,649.19	9,153.70	20,801.89
Buildings and Grounds		46,184.88	5,882.06	52,066.94
Supply Department [3]	87,462.76	86,551.69	2,749.50	89,301.19
"Biological Bulletin"	6,044.42	9,812.79		9,812.79
Administration		24,336.70		24,336.70
Miscellaneous	486.96	1,551.59		1,551.59
	$250,098.72	$253,822.09	$32,806.56	$286,628.65

Total Expenditures	$286,628.65
Total Receipts	250,098.72
	36,529.93
Deduct Carnegie Book Fund Payment	2,671.07
Deficit for Year	$ 33,858.86

None of the totals in the above Statement include any interdepartment charges, nor any charges for depreciation or interest on investment.

[1] Donations were $775 given by the "Associates" of the Laboratory for apparatus, and $169.95 contributed for a Washing Machine.

[2] The Library income of $3,000 is the payment from the Oceanographic Institution towards Library expenses. The monetary value of serials received in exchange for the "Bulletin," estimated at $4,937.80, is not included in the above, nor is the $1,350 received from the Oceanographic Institution for the purchase of books for their account.

[3] The actual sales of the Supply Department were $96,191.85. The values of specimens and supplies furnished Research and Instruction Depts. were $7,132.44 and $4,672.05 respectively. If these values are taken into account and also the gain in inventory of $4,848.29, the increase in accounts receivable of $5,233.85, and a debit charge of $1,800 for administrative and maintenance expense, there would be a net profit of $20,997.70 on the operations of the Supply Department for 1947. This does not take into account the $2,749.50 spent for capital items, or the Auditors' charges of $1,461.67 for depreciation and $2,221.29 for interest on investment. If these had been included, the net profit for the Supply Department would have been $14,565.24.

BIOLOGICAL LABORA
WOODS HOLE
LIBRARY

II. Current Expenses for 1947 by Departments

Administration

Salaries	$ 18,851.55
Central Hanover Bank Trustee Commissions	1,034.87
Falmouth Nat'l Bank Service Charges	142.45
Audit	1,045.83
Treasurer's Office	600.00
Advertising	333.34
Office Supplies	837.74
Sundries (Telephone, Postage, etc.)	1,682.82
	24,528.60
Deduct Cash Receipts	191.90
	24,336.70

Instruction

Salaries and Travel Allowances	7,273.80
Sundries	551.14
	7,824.94

Research (Incl. Apparatus and Chemical Depts.)

Salaries	11,378.87
Travel	200.00
Repairs	798.67
Supplies and Sundries	6,763.96
	19,141.50
Deduct Cash Receipts	1,768.90
	17,372.60

Library

Salaries	10,881.25
Office Supplies	423.29
Sundries	343.65
	11,648.19

Buildings and Grounds

Salaries and Wages	22,630.19
Fuel	2,825.30
Gas	1,871.81
Light and Power	2,749.00
Water	623.88
Insurance	1,666.23
Repairs	10,605.74
Sundries	4,190.39
	47,162.54
Deduct Cash Receipts	977.66
	46,184.88

Dormitories and Apt. House

Salaries and Wages	4,800.50
Lighting, Gas and Water	2,134.87
Repairs to Blds. and Grounds	2,820.28
Outside Rentals	500.00
Laundry	1,390.59
Insurance	734.22
Sundries	1,190.37
	$ 13,570.83

Mess

Salaries and Wages	$ 8,431.22
Cost of Food	21,326.59
Gas, Water, Light and Power	1,650.78
Repairs	231.03
Replacement of Dishes, etc.	391.15
Insurance	603.69
Laundry	311.80
Freight and Express	102.07
Sundries	916.71
	33,965.04

Supply Department

Salaries and Wages	30,756.78
Purchase of Specimens	38,668.92
Chemicals	2,579.60
Containers	3,708.63
Boat Expenses	1,972.44
Truck Expenses	825.69
Freight and Express	3,313.63
Fuel	775.37
Light	96.00
Office Supplies	511.18
Telephone and Telegraph	317.10
Insurance	873.74
Advertising	344.54
Specimens and Supplies purchased for Research	1,058.40
Sundries	749.66
	86,551.69

"Biological Bulletin"

Salaries and Wages	2,101.00
Printing, etc.	7,711.79
	9,812.79

Real Estate (Rented)

Taxes and Insurance on Bar Neck Property (Garage) and Janitor's House	1,002.84

Other Expenses

Workmen's Compens. Ins.	725.28
Truck Expense	222.26
Bay Shore and Great Cedar Swamp Expenses	203.72
Interest on Mortgage	250.00
Evening Lectures	128.96
Special Repairs, 1944 Hurricane Damage	21.37
	1,551.59
Total Expenses	$253,822.09

III. Additions to Capital Assets from Current Funds

A. Land

Improvements Bake House Lot	$ 201.00
	201.00

B. Buildings

Waterproofing of Dormitory	4,000.00
Waterproofing Apt. House	2,780.00
Dormitories	3,349.32
Brick Laboratory	2,116.75
Wharf	450.00
Other Buildings	1,081.14
	$13,777.21

C. Equipment

Apparatus Department	$ 2,127.22
Dormitories	883.93
Mess	1,880.83
Brick Laboratory	1,273.60
Carpenter Shop	347.62
Old Main Building	411.95
Supply Department	2,749.50
	9,674.65

D. Library

Back Sets	2,587.72
Books	801.85
Serials	3,901.46
Reprints	10.76
Binding	1,851.91
	9,153.70
Total Additions	$32,806.56

IV. Gifts for Special Purposes

A. Boat Fund

Contributions Received	$9,335.00
Payments on New Boat	4,032.21
Balance Dec. 31, 1947	5,302.79

In addition securities were received in 1947 from Mrs. W. Murray Crane for the Boat Fund that were subsequently sold for $784.53.

B. Dr. Frank R. Lillie Memorial Fund

Initial Contribution from Dr. G. H. A. Clowes	$1,000.62

V. Real Estate Accounts

A. Devil's Lane Property

Cash Received in 1947 from Sale of Lots		$5,198.00
Disbursements:		
Taxes	$ 286.66	
Road Construction	4,161.66	4,448.32

Cost of Devil's Lane Property to Dec. 31, 1947 was $54,296.13. Eighteen lots were sold in 1946 and 1947 for $14,250.00. $6,990.50 was paid in 1946 and 1947 on these purchases. The Devil's Lane Property as of Dec. 31, 1947, was carried on the books at $40,046.13, with the unpaid installments on the lots sold amounting to $7,259.50 carried as Accounts Receivable.

B. Gansett Property

No cash transactions in 1947 except payment of $87.69 taxes. Gansett Property including Accounts Receivable of $970, and deducting Reserve of $1,950 is now carried on the books at $1,162.36.

VI. Agency Accounts

A. Fellowship Fund

Cash Received from the Lalor Foundation		$ 5,000.00
Disbursements:		
For Fellowships	$2,512.50	
For Apparatus, Supplies and Laboratory Space	1,444.47	3,956.97
Balance, Dec. 31, 1947		1,043.03

B. Cancer Research Account

Cash Received from U. S. Public Health Service as grant-in-aid for "The Mechanism of Cell Division and Protoplasmic Growth" (under direction of Dr. Robert Chambers)	$25,000.00
Cash paid for Salaries, Laboratory Space, Apparatus and Supplies	6,278.81
Balance, Dec. 31, 1947	18,721.19

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1947
(From Auditors' Report)

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 961,036.65	
Securities and Cash in Minor Funds		17,641.02	$ 978.677.67
Plant Assets:			
Land	$ 110,626.38		
Buildings	1,337,188.88		
Equipment	202,358.59		
Library	363,325.72		
	$2,013,499.57		
Less Reserve for Depreciation	722,069.52	$1,291,430.05	
Reserve Fund, Cash		6,218.88	
Book Fund, Securities and Cash		14,301.62	1,311,950.55
Current Assets:			
Cash		18,648.18	
Mortgage Note Receivable		2,425.00	
Accounts Receivable		39,100.94	
Inventories:			
Supply Department	$ 43,932.10		
"Biological Bulletin"	16,775.67	60,707.77	
Investments:			
Devil's Lane Property	47,305.63		
Gansett Property	1,162.36		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Securities	21,464.00		
Retirement Fund	16,388.44	99,020.43	
Prepaid Insurance		5,575.99	
Items in Suspense (Debits)		5,529.88	231,008.19
			$2,521,636.41

Liabilities

Endowment Funds:			
Endowment Funds	$ 959,619.12		
Reserve for Amortization	1,417.53	$ 961,036.65	
Minor Funds		17,641.02	$ 978,677.67
Plant Funds:			
Mortgage Note Payable		$ 5,000.00	
Donations and Gifts	$1,172,564.04		
Other Investments in Plant from Gifts and Current Funds	134,386.51	1,306,950.55	1,311,950.55
Current Liabilities and Surplus:			
Accounts Payable		$ 11,621.59	
Items in Suspense (Credits)		1,937.38	
Reserve for Repairs and Replacements		7,166.71	
Current Surplus		210,282.51	231,008.19
			$2,521,636.41

Respectfully submitted,

Donald M. Brodie,

Treasurer

V. REPORT OF THE LIBRARIAN

1947

The sum of $11,500 appropriated to the library in 1947 was expended as follows: books, $588.11; serials, $3,936.94; binding, $1,851.91; supplies, $444.99; express, $117.52; salaries, $9,475.25 ($3,000 of this sum was contributed by the Woods Hole Oceanographic Institution); back sets, $130.39; insurance, $45.00; sundries, $247.11; total, $16,837.22. The cash receipts of the library totalled: for microfilms, $222.76 (the cost was $531.47); sale of duplicates, $264.20. These receipts revert to the Laboratory; so also do the fees from library readers. There were 55 of these readers in the library during the year.

Of the Carnegie Corporation of New York Fund, $2,457.33 was expended for the completion of 17 back sets and for the partial completion of 12 back set; $213.74 was expended for 10 books.

The Woods Hole Oceanographic Institution budget was $800 plus $500 for additional purchases made during the year. The total spent was $1,322.28. The Woods Hole Oceanographic Institution also contributed $3,000 (see above under "salaries").

During 1947, the library received 1,201 current journals: 327 (13 new) by subscription to the Marine Biological Laboratory; 48 (3 new) to the Woods Hole Oceanographic Institution; exchanges with the "Biological Bulletin," 512 (12 new; 43 reinstated foreign) and 130 (21 new; 3 reinstated foreign) with the Woods Hole Oceanographic Institution publications; 173 (8 new) as gifts to the former and 11

(3 new) to the latter. The library acquired 178 books: 55 by purchase of the Marine Biological Laboratory; 18 by purchase of the Woods Hole Oceanographic Institution; 9 by gift of the authors; 43 by the publishers; and 53 by various donors, those of Dr. Oscar W. Richards (8); Dr. W. J. V. Osterhout (1); Dr. Bradley M. Davis (2) and Dr. Paul S. Galtsoff (2) among the most notable. There were 32 back sets of serials completed; 19 purchased by the Marine Biological Laboratory (17 with the "Carnegie Fund"); 5 by the Woods Hole Oceanographic Institution; 6 by exchange with the "Biological Bulletin"; 2 by exchange with the Woods Hole Oceanographic Institution publications. Partially completed sets numbered 56: purchased by the Marine Biological Laboratory, 29 (12 with the "Carnegie Fund"); purchased by the Woods Hole Oceanographic Institution, 11; by exchange with the "Biological Bulletin," 1; by exchange with the Woods Hole Oceanographic Institution publications, 6; by gift and exchange of duplicate material, 9.

The reprint additions to the library were 6,926; current of 1947, 257; current of 1946, 1,041; and of previous dates, 5,628. Acknowledgment is made to Drs. F. A. Hartman, Charles Packard, and F. K. Knowlton for valuable contributions to the reprint collection. Also, through Dr. E. G. Butler of Princeton University, the reprint collection of Dr. Ulric Dahlgren was presented to the library.

At the end of the year 1947 the library contained 56,594 bound volumes and 149,218 reprints.

In the fall 96 titles of the German journals delayed since 1940 were received in scattered volumes and numbers. The majority of these were for the years 1940–42.

The above report for 1947 is given in a format similar to preceding years. There follows a summary of the main events in the growth of the library from 1924 to 1947 inclusive. The figures of the varying budgets and acquisitions have been transformed for easy reading into graphs covering these twenty-four years. The data, as well as the running account, have been gathered for the most part from the yearly reports of the librarian.

The year 1924 was chosen for the starting point for several reasons. For one, a fair account of the library from its inception in 1888 to 1924 has already been published in the "Collecting Net" of 1929. But more important, the date marks a change in the administration of the library, in its expansion and in its budget. Previous to 1924 there is no mention of a library committee. The librarian (Dr. H. McE. Knower, followed by Dr. R. P. Bigelow) directed the work with the help of a paid assistant from 1914 to 1923. In 1924 a committee was appointed by the trustees with Dr. C. E. McClung as chairman, and Mrs. T. H. Montgomery, Jr. was made the full-time librarian. Dr. McClung remained as chairman through 1924–1931. Professor E. G. Conklin followed until 1941 and Dr. A. C. Redfield served in this office from 1942 to the end of 1947. In 1924, also, the present library was under construction; and in 1925 the collection was moved from room 217 in the Crane Building to its present position. At this time the library acquired a secretary, Miss Deborah Lawrence, whose invaluable assistance has continued throughout the development of the library into the present. The special significance of the period beginning in 1924 consists in the change that occurred in the library budget. Careful checks having been made to list the journals and books necessary for research in an expanding library, large sums of money were secured to purchase these, especially the back sets of needed journals. The accumulated "library fund" of $8,000 was made available for 1924–25. In 1925 the General Education Board contributed $50,000 on the condition that it should be spent for back sets during the years 1926–30. In 1929 the General Education Board gave $200,000 for the general purposes of the library.

In addition to these gifts there have been special funds which came to the library later, not a part of the regular annual budget. The Woods Hole Oceanographic Institution, established in 1930, in that year contributed $5,000 for back sets and books, and for current journals on oceanography. Subsequently its annual budget for this purpose was $1,000 to $600, increased in 1941 to $800. In addition it has contributed to our library salary budget. The appropriation for this purpose was $1,100 in 1944, $1,700 in 1946 and $3,000 in 1947. The "Carnegie Fund" of $25,000 became available in 1941. Since the gift was not conditioned by a time limit for its expenditure and since the market for rare sets has been limited during and after the war years there remains a balance of this fund amounting to about $10,000.

The spacious library, begun in 1924, with five floors of stacks sufficient to hold 100,000 volumes, large reading and cataloguing rooms and librarian's office was thought to be adequate for many years. As early as 1936, however, when the library had acquired half this number of volumes and about 100,000 reprints the librarian reported "it is necessary to explain also how 50,000 volumes (which will be the total in four years if growth occurs at the present rate of 2,000 annually, and and 108,000 reprints if each years adds 3,500) will completely fill space that in 1925 was estimated to be adequate for 100,000 volumes, or 20,000 on each of five floors. The reprint floor at once reduces the available space for volumes to a capacity of 80,000. Besides this the many serial sets and books of quarto size, and over, reduce the space, and half of the bound serials recorded in our count are in reality two volumes bound together, so that the library will at the end of the year 1940 actually be housing more nearly 75,000 volumes, counted as volumes and not by the accession number, and 108,000 reprints." This prediction was amply fulfilled. By 1940 all available space in the stacks and wall shelves was occupied. An appeal to the Rockefeller Foundation for funds to build an addition met with a generous response. This gift of $110,400 was used, during the fall and winter of 1940–41, to erect a new wing which more than doubled the capacity of the library. Special provision was made for readers who can now enjoy private, well-lighted tables.

Through the period from 1924 until 1941 Dr. Frank R. Lillie was President of the Corporation and Chairman of the Executive Committee of the Laboratory. Any analysis of the steps taken in the development of the library throughout these years points directly to his wisdom, forethought and executive ability. He conceived its broad plan and under his guidance the library has grown from a small beginning to its present outstanding position.

The cost of building up and maintaining the library is shown in Figure 1. The total expenditures for each year since 1924 are given, and the amounts used for serials and books, and for back sets. Other expenses, such as salaries, binding, supplies, etc., are not shown. Reference has already been made to the great increase in overall expenditure from 1924 to 1932. The effects of the depression then began to be felt. The income of the Library Endowment and of the Laboratory as a whole dropped sharply. The library budgets have been successively reduced. This is especially noticeable during the war years when foreign journals, those from Germany in particular, could not be delivered and paid for. The extent of the drop in the number of journals received at that time is seen in Figure 2. Beginning in 1944 the number has risen rapidly until now the total is 1,201, as contrasted with 1,339 in 1936. Some of the back numbers published during the war are gradually coming in.

The yearly additions of volumes of serials and reprints is shown in Figure 3. At present there are 56,594 such volumes and in addition 8,000 books. Attention is called to the reprint receipts from 1924 to 1932, an increase of nearly 7,000 each year. During these years the large collections from Dr. Whitman's library and those of Sidney I. Smith and Maynard M. Metcalf were catalogued. At this time also about 5,000 reprints previously listed under books were shifted from the count of volumes to that of reprints. In 1938, besides other generous gifts, a collection from Dr. F. R. Lillie was recorded, and in 1943–1947 Dr. Rudolf Höber's large contribution, those of Drs. E. B. Meigs, W. E. Garrey, A. C. Redfield, Ulric Dahlgren, and others, greatly increased the count.

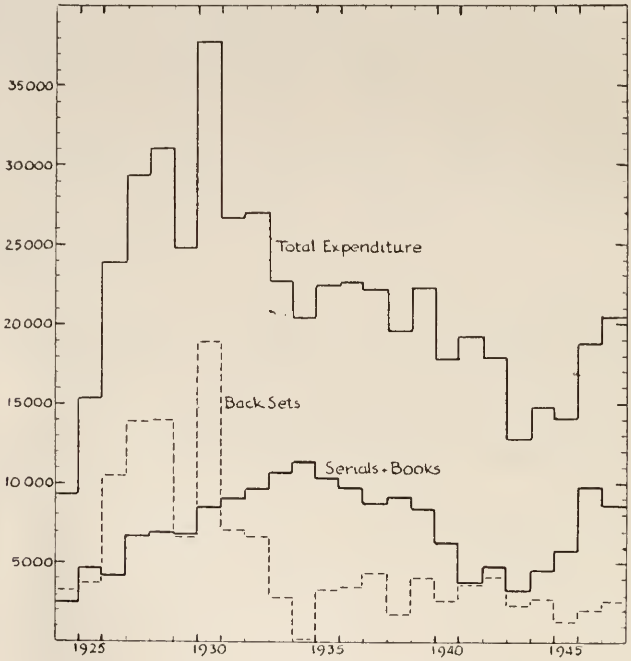

FIGURE 1

Much of the work of the Library Committee and of the staff has already been indicated. But in a summary of the past twenty-four years it is appropriate to note other services that are not as obvious from a study of the tables. That the purchase of journals and books was preceded by a careful selection is self-evident. In the matter of choice the investigators who use the library have always been the arbiters. To aid them in this the librarian, under the direction of the Library

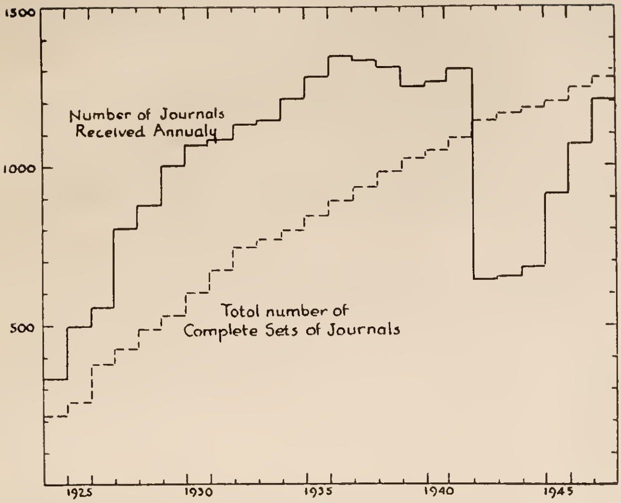

FIGURE 2

Committee has, each summer since 1927, compiled a list of desirable journals and books. The titles on these lists recommended by the investigators, as well as their own suggestions, were obtained either by purchase or exchange. Policies regarding exchange material were decided at the summer meeting of the Library Committee.

Another service rendered by the staff was the preparation of the complete list of journal titles, published as a supplement to the "Biological Bulletin." The full titles and holdings of each journal, with cross references of duplication in titles, were arranged in alphabetical order exactly as they appear on the shelves of the

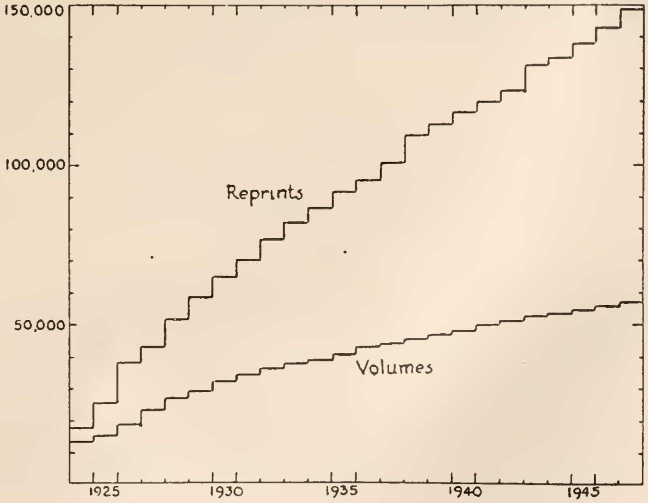

FIGURE 3

library. This made a volume of 80 pages in its initial publication in 1943. Additions, in similar form, have been published in each subsequent year.

For the benefit of the investigators the "sales room" of duplicate reprints and books has been in operation for many years.

The microfilm service, initiated in 1942, has been used extensively by investigators to secure literature not available to them elsewhere. This service has resulted in a reduction in the number of loans requested by outside libraries during the winter months. In this connection the printed list of journals, mentioned above, has been helpful.

The most difficult of the services carried on throughout this period was that of the catalogues. A journal catalogue separated from that of the books and reprints and complete in cross-references proved satisfactory. It seems to present no problem for its future use. On the other hand, the catalogue for books and reprints has always presented difficulties. That the books should be catalogued by subject as well as by author has never been questioned. Whether the enormous collections of reprints should be catalogued in the same way has not met the same unanimity of opinion. Finally the librarian established a system of assigning subjects by which the catalogue became less bulky. By this method a bibliography card was placed in front of each author's catalogued works. On this card a list of his subjects appears and on a subject card a cross-reference is given to the author's name. In this way many names appear as cross-references from the subject, thus eliminating the making of repetitive subject cards. After this system was installed throughout the catalogue, it was estimated that the card count was 472,500. If the new entries following 1947 can be continued in this manner the catalogue should form a useful guide to those unacquainted with the literature of the reprint collection.

This account of the library is not complete without a very special acknowledgment of the constant attention given to its development by the Directors (Dr. Merkel M. Jacobs and Dr. Charles Packard) and by the Library Committee members. All problems, large or small, in policy or in execution, that were laid before them, received attentive guidance and encouragement. Through their suggestions and moral support the work of the library has maintained its growth and stability.

PRISCILLA B. MONTGOMERY,
Librarian (retired)

VI. THE REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY

Gentlemen:

I submit herewith a report of the sixtieth session of the Marine Biological Laboratory for the year 1947.

The Laboratory has suffered an irreparable loss in the death of Dr. F. R. Lillie. For more than forty years he was its guiding spirit; under his leadership it grew from a small, struggling institution to its present eminence. His career began and ended in Woods Hole. Here he came as a young student, and here, full of years and honors, he was buried. For fifty-six consecutive seasons he returned, serving as instructor, investigator, and administrator, always with the interests of the Laboratory at heart. On becoming Director in 1907 he said, "Our purpose is essentially ideal, and its pursuit demands our best efforts and our loyalty." When he resigned in 1926 the trustees wrote, "The Trustees appreciate the conspicuous ability, combined with unselfishness, with which you have guided the Laboratory. To you, in large measure, is due the steady broadening in scope and method that has been so conspicuous a feature of its work in recent years. As its Director you began with an institution already rich in achievement but still poverty stricken in respect to material things. You have left it unsurpassed in equipment and endowment, the center of activities that exert an always increasing influence on scientific progress throughout the world."

In his reply Dr. Lillie characteristically gave all credit to others—to the Trustees, to Mr. Crane, to the technical staff, and added, "Patience and faith were the only other necessary factors. I have no fear for the future as long as the Woods Hole spirit survives." Genuine cooperation and mutual helpfulness, he said, constitute the basis of this spirit. But elsewhere he added this note of warning, "We must not allow ourselves to forget that the principles for which we stand are never entirely won."

Those of us who worked with him will not forget his quiet and unhurried approach to current problems, his ability to foresee future needs, his untiring and successful efforts to meet them; and his lifelong devotion to the welfare of this Laboratory. It is fitting that a tablet to his memory should be placed in the entrance of the building. But more than that, we should build an F. R. Lillie Laboratory which will remind future generations of biologists of our debt to him.

1. *Changes in Personnel*

Mrs. Montgomery, our Librarian since 1924, retired at the end of 1947. For twenty-three years she carried on the business of the Library, beginning at the time when it was a small and insignificant collection, and continuing throughout the entire period of its expansion. In addition to her regular Annual Report she has presented a brief history of the Library during her incumbency. Of her own important and successful work she says little. But all who use the Library are fully aware that she, more than anyone else, is responsible for its completeness, its fine arrangement, and its perfect condition. We owe her a debt of gratitude for her outstanding work in the development of this world famous part of the Laboratory.

Miss Deborah Lawrence, who has worked with Mrs. Montgomery since 1925, has been appointed Acting Librarian. Under her supervision the Library continues to be carried on in a most satisfactory way.

Mr. Larkin, Superintendent of Buildings and Grounds, also retired this year. Through a long term of service he has been devoted to the interests of the Laboratory, on hand in emergencies at any time of day or night, always capably carrying on the work of his department. He has been retained as Consultant because of his intimate knowledge of the complicated installations in our buildings.

Mr. Robert Kahler, for many years Mr. Larkin's assistant, is now in charge of the plant, and has demonstrated his competence and resourcefulness in meeting the usual and unusual problems connected with the work.

Mr. Homer P. Smith joined the staff in July 1947 as Assistant Business Manager. The need of a second man in the office has been felt for some time for the business of the Laboratory has increased greatly in recent years, and one man could not be expected to carry the entire burden. Under Mr. MacNaught's tutelage he has become familiar with the various phases of work, has adapted himself to the situation, and has proved to be a valuable addition.

2. *New Boats*

The Laboratory is now well provided with boats for all its various needs. New craft were urgently needed to replace the *Sagitta,* considered unsafe after 40 years of service, to collect the large amount of material required by schools and colleges, and to carry the classes on their field trips. It was, therefore, decided to make an appeal for funds to all members of the Corporation, to all workers at the Laboratory, and to friends living in the vicinity of Woods Hole. The Boat Fund Committee, under the direction of Dr. Heilbrunn, was highly successful in obtaining contributions, the total amount being $10,035. Among the nearly 300 donors were the Lilly Endowment $3,000, Mr. William Proctor $1,000, and Dr. G. H. A. Clowes $1,000.

Dr. Redfield, Chairman of the Committee appointed to secure a suitable boat, after visiting a number of boat yards, advised that the new craft should be designed by Mr. Crocker, a naval architect. After the Committee had approved the plans, construction was begun and carried forward without delay. The boat, 33 feet on the water line, has a cruising speed of 12 knots, and is equipped with dredging gear and a two-way radio. It will be in service in the summer of 1948.

In the spring of 1948 Mr. Crocker reported that a much larger boat, which he designed, could be bought for $12,000—about half the original cost. Mr. Walter Kahler inspected it thoroughly and urged that the Laboratory acquire it. The Executive Committee approved its purchase. The boat, large enough to accommodate the entire Zoology class, will be used during the summer of 1948.

3. *Repairs and Improvements*

In the course of the past two years we have made many repairs and improvements in several of our buildings. The much needed waterproofing, roof repairs, and outside painting of the Brick Building have been completed. The Apparatus Department is now situated in the well lighted and airy basement of the new wing of the Library. The rooms which were vacated will be now used as laboratories. The Chemical Department now has an air-conditioned room where special measurements can be made. The south wing of Old Main has been shored up so that the floors no longer vibrate as freely as in the past. Useful changes have been made in the Botany and Rockefeller Buildings. In the Stone Building a freight elevator is under construction. The top floor can be used for the storage of Supply Department materials. The Drew House has been put into good shape and painted; a new apartment was made out of the reception room of the Apartment House. Here also extensive repairs to the balcony supports were imperative. These and other much needed changes and repairs have cost nearly $38,000.

We must next make extensive alterations in the Supply Department Building, and put in good condition the wooden residences. Each year at least one of our buildings should be restored to first class shape. In this way they can be prevented from falling into serious disrepair.

4. *The Housing Problem*

Each year since the war ended we have experienced great difficulty in providing living quarters for those who want to work here. This is, to a considerable extent, due to the fact that, compared with former years, many more of the young investigators and students are married and require apartments, suites, or other special accommodations for their growing families. The Laboratory can provide for a relatively small proportion of these applicants; in the village there is a reluctance to rent rooms to families with children. As a result a number of good investigators are forced to withdraw their requests for research space.

When the housing situation became critical soon after the Brick Building was finished, Dr. Lillie urged the erection of a building to accommodate a large number of single investigators, and, in addition, ten bungalows for families. Today we can provide for the first group, but for the latter we need more apartments or small houses. To add to our present Apartment House or to erect a new one is too costly a venture at this time. But simple houses could be built on Devil's Lane property at a moderate cost. I believe that if the Laboratory built several, with a view to selling them to our members on easy terms, we could soon dispose of them, together with the lots on which they stand. Without doubt such a housing project would stimulate others to purchase Devil's Lane lots. Thus both the workers and the Laboratory would be benefited.

5. *Lalor Fellowships*

Lalor Fellowships, established by the Lalor Foundation, were granted to the following investigators:

Senior Fellow:	Prof. Jean Brachet, University of Brussels, Visiting Professor at the University of Pennsylvania
Junior Fellows:	Dr. I. M. Klotz—Northwestern University
	Dr. Arnold Lazarow—Western Reserve University
	Dr. Benjamin Libet—University of Chicago
	Dr. Claude Villee—Harvard University

6. *Winter Research*

The Laboratory of Experimental Cell Research, under the direction of Dr. Robert Chambers, has been engaged since the Fall of 1947 in the study of the mechanism of cell division and growth, employing micromanipulation methods and tissue cultures.

7. *Gifts*

The Laboratory gratefully acknowledges the following gifts:

The Associates of the Marine Biological Laboratory, $775
Dr. G. H. A. Clowes—for a Lillie Memorial, $1,000
Mrs. W. Murray Crane, $700
Contributors to the Boat Fund, $10.035

8. Election of Trustees

At the Meeting of the Corporation on August 12, 1947, the following trustees were elected:

Class of 1951

W. C. Allee
C. L. Claff
G. H. A. Clowes
K. S. Cole
P. S. Galtsoff
L. V. Heilbrunn
J. H. Northrop
A. H. Sturtevant

9. There are appended as parts of this report:

1. Memorials to Prof. William B. Scott, Prof. Robert A. Harper, Mr. George M. Gray, Professor Herbert S. Jennings, Professor Samuel O. Mast, and Professor L. L. Woodruff
2. The Staff
3. Investigators and Students
4. Tabular View of Attendance
5. Subscribing and Cooperating Institutions
6. Evening Lectures
7. Shorter Scientific Papers (Seminars)
8. Members of the Corporation

Respectfully submitted,

CHARLES PACKARD,
Director

1. MEMORIALS

William Berryman Scott, 1858–1947

By E. G. Conklin

William Berryman Scott, the oldest member of the Board of Trustees of the Marine Biological Laboratory, was born on the birthday of Charles Darwin and Abraham Lincoln, February 12, but 49 years later than these great predecessors, viz. 1858. He died in his 90th year on March 29, 1947 in Princeton, New Jersey, his life-long home.

He and his colleague at Princeton, William Libbey, first visited Woods Hole in 1883 on invitation Spencer F. Baird, head of the U. S. Fish Commission, to confer with him on plans for the development of the Fisheries Station at Woods Hole, and as a result, Libbey contributed in the name of Princeton University, $1,000, toward the purchase of the land on which the Station was established.

In 1890, two years after the founding of the Marine Biological Laboratory, Prof. Scott became a member of the Corporation and in 1897 he was elected a Trustee and continued in that office and as Trustee Emeritus until his death. During these forty years as Trustee, he made it a point of honor to be present whenever possible at the annual meetings, and in 1897 and 1898, he gave lectures at the Laboratory on the methods and results of his paleontological researches.

Although he was for fifty years a member of the staff of the Department of Geology at Princeton, and for forty-six years head of that department, he was primarily a zoologist. At the close of his senior year in college, he and two other classmates, Osborn and Speir, organized a scientific expedition to Colorado and Wyoming. The collection of vertebrate fossils made that summer of 1877 was described in their first scientific publication and was instrumental in shaping the future careers of Scott and Osborn. After a year of graduate study at Princeton and a second expedition to the West in the summer of 1878, Scott spent two years in graduate study in Europe; first with Huxley in London, then with Balfour in Cambridge, and finally with Gegenbaur in Heidelberg. His work with these masters was in anatomy and embryology. Under the stimulus of Balfour, he, with Osborn, completed and published a study on "The Early Development of the Common Newt," the first such study on the embryology of a urodele. In Gegenbaur's laboratory he was assigned for study the valuable material which had been collected by Dr. Calberla, deceased, on the embryology of a cyclostome fish and the results of this study were published in Gegenbaur's Morphologisches Jahrbuch in 1880 as his thesis for the Ph.D. degree, with the title, "Beitrage zur Entwicklungsgeschichte der Petromyzonten."

Thus at the early age of twenty-two years, he had finished his work for the doctor's degree, published three important papers, been a leader in two exploring expeditions, and had met on terms of intimate friendship and cooperation, some of the foremost scientists and scholars of Europe and America.

On his return from Europe, he was appointed assistant in geology at Princeton and three years later was made full professor in that department, which position he continued to hold until he had completed fifty years of teaching at Princeton University. During that time, he made eight additional exploring expeditions to the West and published more than 150 paleontological reports. Perhaps his most monumental work was the "Reports of the Princeton Expedition to Patagonia" which was published in nine magnificent volumes, of which he was editor and co-author. His later work, undertaken after he was seventy-six years old, in association with his former student and colleague, Dr. Jepson, was a great monograph of 1,000 pages and 100 plates on the "Mammalian Fauna of the White River Oligocene"; while his latest work was a similar monograph on the "Mammalian Fauna of the Uinta Formation," upon the final pages of which he was at work until two days before his death.

In addition to these research publications, he was the author of a number of important books of a more general nature, among them, an "Introduction to Geology," which ran through three editions. "A History of Land Mammals of the Western Hemisphere," two editions; "Physiography, the Science of the Abode of Man"; "The Theory of Evolution"; and finally, a most interesting and important autobiography, "Some Memories of a Paleontologist."

He was abundantly honored both in Europe and America by universities and learned societies. The Universities of Pennsylvania, Harvard, Princeton and Oxford, gave him honorary degrees. He received ten medals and awards from learned societies here and abroad. He was elected a member of the American Philosophical Society when he was twenty-eight years old and at the time of his death, had been a member for more than sixty years. For seven years he was president of that society of which his great, great, great-grandfather, Benjamin Franklin, had been founder and first president. He was also president of the Geological Society of America (1925) and of the Paleontological Society (1911), and a member of the National Academy of Sciences, the American and British Associations for Advancement of Science, the American Academy of Arts and Sciences, the Academy of Natural Sciences of Philadelphia, the New York Academy of Sciences, the Washington Academy of Sciences, the Geological, Zoological and Linnean Societies of London.

Professor Scott was a brilliant lecturer and he often enlivened scientific meetings with his humorous stories and his phenomenal memories of great men and events. Nevertheless, he was a scholar and thinker rather than a popular lecturer or writer. Fortunately, he has recorded in his autobiography many of his memories of some of the greatest men of his generation.

The Corporation and Trustees of the Marine Biological Laboratory record their sorrow in the loss of one of their oldest and most distinguished members and transmit to the members of his family this expression of their esteem and sympathy.

Dr. Robert A. Harper

By Edmund W. Sinnott

In Dr. Harper's death, plant science has lost a man who for years was one of its greatest leaders.

Dr. Harper was born on January 21, 1862 at Le Claire, Iowa. He received his B.A. at Oberlin in 1886 and then for two years taught Latin and Greek at Gates College. From 1889 to 1891 he was instructor in science at Lake Forest Academy. He took an M.A. at Oberlin 1891 and then served for a time as professor of botany and geology at Lake Forest College.

Dr. Harper's chief interests centered more and more in botany and he soon determined to make this his career. As so many young botanists did in the nineties, he went to Germany for graduate work. At Bonn he came under the influence of Strasburger and other notable teachers, taking his doctorate in 1896. Here began his life-long interest in cytology. Soon after his return to America, he went to the University of Wisconsin where he became professor of botany and head of the department. In 1911 he was called to Columbia University as Torrey Professor of Botany, serving until his retirement in 1930. Here he reorganized the department and greatly widened its scope and activities. He was keenly interested in the New York Botanical Garden, the Boyce Thompson Institute for Plant Research and the Torrey Botanical Club.

Dr. Harper was a student of plant cells since his days at Bonn, but he was much more than a mere cytologist. He liked to describe himself as a cellular physiologist and studied many aspects of the activities of all sorts of plant cells. He was for a long time especially concerned with a study of reproduction in the fungi, and his work on nuclear behavior in the ascomycetes is classic. Studies of development and morphogenesis particularly appealed to him and he was a stout supporter of the view that the phenomena of development are best approached through a knowledge of the behavior of cells. He was much interested in some of the simpler algae, notably Pediastrum and Hydrodictyon, in which he investigated the problems of cellular activities.

Unlike so many biologists of today, Dr. Harper had a wide knowledge not only of his own science, but of others and of wider fields of learning. He was a good field botanist, a successful farmer, a skilled experimenter, and a man of wide reading and erudition. He encouraged his students to train themselves broadly and not to be carried away by the fashions of the time. He was a stimulating teacher, and in a discussion, delighted to take the less popular side and to defend it vigorously. He was highly critical and a foe of slipshod works. His own papers were beautifully done and his drawings, in particular, were remarkably fine.

Dr. Harper had close contacts with Woods Hole for many years. He was a student here in 1891, along with Bradley Moore Davis, C. P. Sigerfoos and Katherine Foote. In 1893, he worked as an investigator at the Laboratory. Frequently during ensuing years he came to Woods Hole for part of the summer. He was elected to the Board of Trustees in 1911 and in 1932 became Trustee Emeritus. In recent time he has rarely come to the Laboratory but devoted all his time to his farm at Bedford, Virginia, where he spent his last years happily close to the soil and with plants he loved.

Dr. Harper's warm and friendly personality endeared him to his many students and to a host of friends all over the world. He was a great teacher, a friendly opponent in debate, and a constant stimulus and inspiration to all who knew him. He will be sorely missed.

George M. Gray

By W. C. Curtis

George Milton Gray was born at Bristol, Rhode Island, November 2, 1856. As a boy he was interested in natural history, particularly in birds and insects. When a young man he worked as a taxidermist, gave lectures on birds at a boys' camp and made collections in the vogue of his day. He was thus a naturalist from his youth up. Later, he was discovered by Dr. H. C. Bumpus and engaged as a technician in the Department of Zoology at Brown University. Mr. Gray first came to the Marine Biological Laboratory in the summer of 1891 as a laboratory assistant. He served in this capacity for six summers (1891–96), as collector for two summers (1897–98), and became Curator of the Supply Department in 1899.

Although this department was established in 1891, its effective operation began with Mr. Gray. At first the orders were filled only in the summer months. In the years 1896–97 and 1897–98, a stock was shipped to Williams College, and sales totaling about $125 per year were made during each of these winter periods. The trustees were elated when it was announced that the sales might exceed $500 for the summer of 1897 and again in 1898. In September 1899, when Mr. Gray became Curator and a year-round appointee of the Laboratory, the department began its continuous existence at Woods Hole. It was then located in the basement space between the two wings of the Old Main Building. In winter the supplies were moved to the invertebrate laboratory where a stove was set up to keep Mr. Gray and the specimens from freezing. The Stone Building, to which the department was transferred several years later, was luxury indeed when he was first established there. As a result of his untiring efforts during these early years, the department flourished. In the year 1912 the sales totaled $13,966.35. In 1925 the total was

$57,771.67. The creation of this necessary adjunct to the work of the Laboratory and important source of revenue was largely the work of George M. Gray.

Mr. Gray not only established the reputation of the department for reliability and for quality of material, he also established a record of service to classes and investigators. Beginning as a one-man organization, it employed an increasing number of assistants, and the role of those who "worked in the Supply Department" for a summer or two and later attained distinction in some field of biological science is an impressive one. Another notable contribution by Mr. Gray was his personal influence upon these youngsters.

In later years he was able to devote more time to aspects of the work in which he was particularly interested. After he withdrew from the Supply Department (1931), he became Curator of the Museum, which was named in his honor and was his special pride. He was fairly active even as Curator Emeritus since 1935. He died December 1, 1946 and is buried in the Woods Hole Cemetery.

Writing in a laboratory room, the windows of which look out upon the Hole and the islands where I worked as his assistant fifty years ago this summer, I cannot forbear my personal tribute. I have always thought him one of the most honest and kindly men I ever know. He was my close friend always from that summer long ago. About that time I was taken by a remark, made sadly by an elderly clergyman wise in the ways of men, that he had known some individuals to whom he thought the term "Christians" might be applicable although he had never seen any reason for applying it to church members as a group. I thought then and I have thought ever since that George Milton Gray was one of the few among my acquaintances to whom I would apply that term. We of the Marine Biological Laboratory never had a more devoted service nor greater loyalty than he gave us. He well exemplified the dictum of his faith: "He that would be great among you let him be the servant of you all."

Dr. Herbert Spencer Jennings

By O. C. Glaser

With the death of Herbert Spencer Jennings, at Santa Monica, California, on April 14, 1947, the Marine Biological Laboratory lost one of its most distinguished members—a trustee for 33 years and a trustee emeritus since 1938.

The son of a physician, Jennings was born in Tonica, Illinois, on April 8, 1868, and educated as he said "in most states of the Union." This migratory life continued with only short interludes until he was 38. Like his namesake, he was precocious and for years hard pressed by economic difficulties and the struggle for scientific opportunity. At 20 he prepared for college, so to speak, as an Assistant Professor of Botany and Horticulture at the Agricultural and Mechanical College of Texas. As an undergraduate at Ann Arbor, he joined the Biological Survey of the Great Lakes conducted by the Michigan State Board of Fisheries and laid the foundations for his first monograph on Rotifers, published in 1894—a year after receiving his B.S. from the University of Michigan. At Harvard he received the M.S. in 1895 and his Ph.D. in 1896. His thesis on cell-lineage of the rotifer Asplanchna herrickii, related the orientation of the spindles to the general problems of developmental mechanics whose solution he projected into the molecular realm.

Travelling fellowships enabled Jennings to spend the following year with Max

Verworn in Biedermann's Laboratory at Jena. Here began his preoccupation with the reactions of unicelluar organisms.

Returning in 1897, he became Professor of Botany and Bacteriology at the Agricultural College of Montana; accepted next instructorships, first at Dartmouth, then at Michigan where he became Assistant Professor of Zoology in 1900 and remained until called in 1903 to an Assistant Professor at the University of Pennsylvania.

These frequent translocations must have had serious disadvantages but his tremendous drive and unbounded enthusiasm found or created time for both teaching and research. By 1906 when he left Pennsylvania, he had to his credit at least 40 publications. The joint text with Reighard on the Anatomy of the Cat based on his own dissections and illustrated by Mrs. Jennings, belongs to the second Michigan period. Here too, as Director in 1901 of the Biological Survey of the Great Lakes under the U. S. B. F., he gathered material for three additional monographs on the Systematics of Rotifers and in connection with his teaching gave the first expression to a lively and recurrent interest in simulacra. Outstanding among the special contributions were his Psychology of the Protozoa in which Paramecium is shown to have "hardly taken the first step in the evolution of mind," and his analysis of the Biological Significance of Asymmetry. There were further studies on stimulation in Protozoa, followed by more from Pennsylvania. There were also publications on the behavior of the earthworm, the sea-anemone and the starfish—the latter a veritable storehouse of information on the activities of this animal—appearing in 1907. Many of his results during the Philadelphia period were either summarized in the great monograph on the behavior of lower organisms published by the Carnegie Institution or in the famous Behavior of the Lower Organisms appearing in the Columbia Biological Series in 1906; reprinted in 1915; and translated by Ernst Mangold in 1914 into German. The chief product of all his meticulous observations and simple experiments was a general outlook with variability, modifiability, unity and adaptiveness of behavior as the central themes.

With his transfer in 1906 to an Associate Professorship of Experimental Zoology at the Johns Hopkins University and his subsequent elevation to the Henry Walters Chair of Zoology and Directorship of the laboratory—both in succession to William Keith Brooks—Dr. Jennings entered upon his only long tour of duty at any one institution. He also changed his field of investigation. Although theoretical and controversial writings on behavior continued to appear, 1908 marks the beginning of a long series of researches on the life-cycles, heredity, variation, and evolution of Protozoa, notably Paramecium, Arcella, and Difflugia. In 1928, he reverted to his rotifers and, with Ruth Stocking Lynch, published two papers on Age, Mortality, Fertility, and Individual Diversities in Proales sordida.

During his genetic phase, Jennings substituted for full verbal description, long tables of measurements and enumerations. He became a biometrician. So highly did he perfect his mathematical techniques and insights that he was called upon to act as a statistician for the Food Administration during the first World War. Among his most important genetic results we must cite the analysis of conjugation in Paramecium whose significance he found in the diversities so created rather than in any rejuvenating effects; his pure lines in the same organism; and finally, the discovery of mating types—at long last the key to Protozoan Genetics. Throughout this period, Jennings strengthened his position as the apostle of diversity. He also developed further his inborn sensitivities to the more general intellectual and social climate. Contributions on special and general methodology such as genetic method and Radical Experimental Analysis appeared in a stream of critical evaluations of Vitalism, Mechanism, Determination and Freedom. In the social area, he wrote on Immigration, Defectives, "Undesirable Aliens," Racial Progress, the Family and Marriage. Apparently unaware of his powers, he invaded more distant territory. In his essay on the advantages of Growing Old, the euphorious state created by the presentation of a portrait of one's self and the pessimistic outlook of a young man trying to lead the life of a productive scholar on a $900 instructorship, are contrasted with humor, pathos and artistry sufficient to suggest pure literature. All told, the output of 32 years at Johns Hopkins amounts to about 120 papers, long or short, and seven books.

Modest, shy, nervous and frail, Jennings nevertheless accepted many outside lecture engagements. He spoke with great animation and charm to audiences invariably responsive to his sincere excitement and well ordered presentations. He gave more than one of our evening lectures. Few who heard his mathematical analysis of the data on genic linear array will forget his enraptured delight with the Naperian Case or the suspense he created and maintained until the final unveiling of Morgan's own theory at the very end. He gave the Terry lectures at Yale; the Vanuxem, at Princeton; the Leidy, at Pennsylvania, where he received the first award of the Leidy medal.

In 1931–32 he was Visiting Professor at Keio University, Tokio, and in '35–'36, Eastman Visiting Professor at Oxford. After retirement, he gave the Patten lectures at Indiana; became Visiting Professor for one year and remained as Research Associate at the University of California in Los Angeles.

Dr. Jennings was active on the Editorial Boards of four Journals; was a member of the National Academy, the American Philosophical Society and other coveted American Academies. He was President of the American Society of Zoologists (1909). His presidential address (1911) to the American Naturalists on Heredity and Personality—one of the most memorable of his gems—exposed, with a gaiety born of many insights, the genetic and environmental odds against the birth of any particular individual. Foreign recognitions included Honorary Fellowship in the Royal Microscopical Society, Corresponding Memberships in the Russian Academy of Sciences and in the Société Biologique de France or de Paris—he was not certain which. His honorary degrees proved quite unmanageable. The British Who's Who for 1944 records eight such degrees; the American counterpart and the American Men of Science both also in 1944 each list six. Agreement on totals, however, obscures the true diversities among four D.Sc.'s, three LL.D.'s and an Oxford A.M.; one D.Sc. and five LL.D.'s; and four D.Sc.'s with two LL.D.'s. One highly reputable LL.D. he avoided. As long as there were Paramecia to measure, count and keep in order, why bother about honorary degrees?

Whoever recalls him will continue to regret that his work at the Marine Biological Laboratory ended in 1933 and that he was a regular attendant only during the decade of the twenties. We should have liked to share him with many others. His inspiring example and achievement can be fully appreciated only against his background of almost continuous ill health. Yet he was always friendly and cheerful; always excited about something; and ready to discuss a case, not for the sake of argument, but because of his passions for both sides of every question; for clarity of mind and for fairness. Impelled as he was by ceaseless cerebration, he nevertheless lists two related forms of recreation—travel and motoring. As a travelling companion, one can hardly imagine another more delightful; however, his friends who either rode with him or merely observed him spiraling briskly down the main street of Woods Hole were far too apprehensive to benefit from the recreative powers of his driving.

Samuel Ottmar Mast

By B. H. Willier

On October 5, 1871, Samuel Ottmar Mast was born on a farm near Ann Arbor, Michigan. His early schooling, academic training and teaching experience were in his native state. After obtaining a "full diploma" in 1897 from the Michigan State Normal College, he went to the University of Michigan where he received the B.S. degree in 1899. From 1899 to 1908, he was Professor of Botany and Biology at Hope College, Holland, Michigan. In 1906, he received the Ph.D. degree in zoology from Harvard University. He then came to the Eastern seaboard where he spent the remainder of his life. For a period of three years (1908–1911) he was a member of the biology staff at Goucher College. In the autumn of 1911, he joined the zoology staff at Johns Hopkins as associate professor which soon culminated in a Professorship of Zoology and later upon the retirement of the late Professor H. S. Jennings in the chairmanship of the Department of Zoology (1938–41). Since 1942, he has been professor emeritus of zoology.

According to the records, he first attended the Marine Biological Laboratory during the summer session of 1907. In 1908, he was elected a member of the Corporation and later (1936–1942) served as a member of the Board of Trustees and of its executive committee for two years (1938–1940). Since 1942 he has been Trustee Emeritus. He and his family have been regular summer residents of Woods Hole for a period of over twenty years. Over these years, he has been in regular attendance as an investigator and his interest in the laboratory has been constant and genuine.

In 1908, he married Grace Rebecca Tennent, the sister of the late David Hilt Tennent of Bryn Mawr and of this laboratory. She and three daughters and many grandchildren survive him. He died quite suddenly on Monday evening, February 3, 1947, at the age of 75 at his home in Roland Park in the city of Baltimore.

The life long work of Professor Mast was directed toward an understanding of the physiology of the "lower" organisms, especially the Protozoa. His major interest was in the mechanisms of behavior of these forms and more specifically in their reactions to light. This is best exemplified in his most significant book, "Light and the Behavior of Organisms" (1911) and in his numerous published papers on the motor responses, factors in the process of orientation, etc. of a variety of unicellular animals and other invertebrates. In 1926, as a result of his interest in the behavior of Amoeba proteus he formulated a theory to account for amoeboid movement, which has received wide recognition. His wide interest in the physiology of the Protozoa led him later to make a study of the nutrition of the colorless flagellate, Chilomonas. Together with Dr. Donald M. Pace and other students, he showed that this organism in the total absence of light can synthetize carbohydrates, fats, proteins and protoplasm from a few simple inorganic salts, resembling in this respect, the green plants and certain bacteria. During the last few years of his life, Mast turned his attention to an investigation of the processes of feeding and digestion in the Protozoa, which culminated in his most significant paper on this subject entitled, "The Food-Vacuole of Paramecium." This work is a fitting and lasting example of the exactness and care which characterized all of his researches and publications. His every publication was marked by the meticulous care with which each word and phrase were weighed to make sure they meant exactly what he had in mind, no more and no less.

Professor Mast commanded to a marked degree the loyalty and admiration of graduate students. He had many pupils and has trained a whole generation of zoologists who have much to thank him for. His loss is deeply felt by many friends, former colleagues and students, and no less by the community of biologists at Woods Hole.

Dr. Lorrande Loss Woodruff

By R. G. Harrison

Lorande Loss Woodruff, Colgate Professor of Protozoology at Yale University and Director of the Osborn Zoological Laboratory, died at his home in New Haven after a long illness on June 23, 1947 in his 68th year. With his passing, the Corporation of the Marine Biological Laboratory loses a member of more than forty years standing and the Board of Trustees, one who had served faithfully for 24 years.

Dr. Woodruff was born in New York on July 14, 1879, and received his education in his native city, graduating from Columbia University with the A.B. degree in 1901 and the Ph.D. in 1905. Before completing his graduate work, he was appointed Assistant and later Instructor in Biology at Williams College, where he remained until he was called to Yale in 1907. There he served successively as Instructor, Assistant Professor and Professor, until his death. He became Chairman of the department and Director of the Osborn Zoological Laboratory in 1938, but took leave of absence in November 1946 on account of ill health.

His connection with the Marine Biological Laboratory began in 1905 when he attended the summer session as Investigator and Instructor in the Invertebrate Course and was elected to membership in the Corporation. Four years later, he joined the Staff of the course in Embryology of which he remained a member until 1914. During the absence of Dr. Calkins in the summer of 1927, he was in charge of the course in Protozoology. Elected to the Board of Trustees in 1923, he served with them until his death and during the years 1930–32, he was a member of the Executive Committee.

Coming to Yale at a time when a radical reorganization of the instruction in biology was to be undertaken, Woodruff took part from the first in teaching general biology and in 1910 assumed full charge of the general course in Yale College. This he built up into one of the soundest and at the same time, most popular courses in the University. Through the years, thousands of students listened to his masterly lectures, later incorporated in his textbook, "The Foundations of Biology," which has been very widely used and has run through six editions.

Woodruff's research was exclusively in the field of unicellular organisms. Beginning with his doctoral dissertation, which was done under the direction of the late Professor Calkins and published in 1905, he made many contributions to our knowledge of the life history of ciliates, their division rate, nuclear reorganization, the effect of environmental factors on their life cycle. He is perhaps best known for the famous pedigreed race of Paramecium aurelia, which was carried for eight years with daily isolation of the products of division, thus precluding conjugation and showing that these organisms can reproduce asexually indefinitely without dying out. In the first eight years over 5,000 generations were obtained and afterward the culture was carried in a less rigorous manner though sufficiently carefully to exclude conjugation except possibly between closely related individuals. The culture has now reached more than 24,000 generations without loss of vigor. In the course of this work, Woodruff and Erdmann discovered that, corresponding to the rhythms in division rate, the nuclei of the paramecia undergo a reorganization process which they termed "endomixis" and which they described as a form of nuclear reorganization with syncaryon formation. This stirred up much discussion and more recently the process has been described by others as autogamy, involving fusion between micronuclei from the same cell.

His research naturally attracted graduate students, and throughout the years many have written their dissertations under his direction and carried his methods to new centers, just as his assistants and students in the course in general biology, many of whom have become teachers, have spread his ideas of the teaching of biology throughout the land.

Woodruff was intensely interested in the history of science. Early in his career at Yale, he organized a course in the history of biology which he continued throughout his life. He was a collector of scientific books of historical significance. He published a number of essays and addresses in this field and organized two series of lectures on the history of science under the auspices of Gamma Alpha Fraternity, which were later published in book form under his editorship. A paper on "Baker on the Microscope and the Polypa" led to a friendly encounter with a descendant of Trembly, the famous author of the treatise on Hydra published in 1744.

Woodruff was Chairman of the Division of Biology and Agriculture of the National Research Council, 1928–29. He was a member of many scientific societies, including the National Academy of Sciences, the American Society of Zoologists, of which he was Secretary-Treasurer, 1907–09 and President in 1942, the American Physiological Society, the American Society of Naturalists (Vice-President, 1923), the American Association for the Advancement of Science, (Fellow) and others. He was a member of Phi Beta Kappa, Gamma Alpha and Sigma Xi, having been President of the Yale Chapter of the last in 1915. He lectured on Protozoology at four summer sessions of the Mountain Laboratory of the University of Virginia. For two terms of three years each, he was an Associate Editor of the *Journal of Morphology*. In 1935, he received the Townsend Harris medal from the College of the City of New York, where he had been as a student before entering Columbia.

For one who was closely associated with Woodruff for nearly forty years, it is difficult to realize that this intimate relation has been forever broken. The association was one of mutual trust throughout and without serious disagreement. He was always on the side of high standards, which he applied to himself as well as to others. Indeed, this was one of his outstanding qualities, as was his intense loyalty to the institutions he served. He was quiet and reserved, but with a kindliness that meant much to those about him. With all of his reserve, he could be deeply moved, and he never recovered from the shock of Mrs. Woodruff's death, which came with such cruel suddenness in March 1946.

The members of the Corporation of the Marine Biological Laboratory desire to record their sorrow over the loss of one of their body, a friend and a fellow servant whom they will miss acutely and whose memory they will always cherish.

2. The Staff, 1947

Charles Packard, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

Senior Staff of Investigation

E. G. Conklin, Professor of Zoology, Emeritus, Princeton University.
*Frank R. Lillie, Professor of Embryology, Emeritus, The University of Chicago.
Ralph S. Lillie, Professor of General Physiology, Emeritus, The University of Chicago.
A. P. Mathews, Professor of Biochemistry, Emeritus, University of Cincinnati.
G. H. Parker, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. Consultants

T. H. Bissonnette, Professor of Biology, Trinity College.
Libbie H. Hyman, American Museum of Natural History.
A. C. Redfield, Woods Hole Oceanographic Institution.

II. Instructors

F. A. Brown, Associate Professor of Zoology, Northwestern University, in charge of course.
W. D. Burbanck, Professor of Biology, Drury College.
C. G. Goodchild, Professor of Biology, S.W. Missouri State College.
John H. Lochhead, Professor of Zoology, University of Vermont.
Madelene E. Pierce, Associate Professor of Zoology, Vassar College.
W. M. Reid, Associate Professor of Biology, Monmouth College.
Mary D. Rogick, Professor of Biology, College of New Rochelle.
T. H. Waterman, Instructor in Biology, Yale University.

III. Laboratory Assistants

Virginia L. Fogerson, Vassar College.
Amos L. Hopkins, Harvard University.
Marie Wilson, Western Maryland College.

EMBRYOLOGY

I. Consultants

P. B. Armstrong, Professor of Anatomy, College of Medicine, Syracuse University
H. B. Goodrich, Professor of Biology, Wesleyan University.

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* Deceased.

II. INSTRUCTORS

Donald P. Costello, Professor of Zoology, University of North Carolina, in charge of course.
Howard L. Hamilton, Assistant Professor of Zoology, Iowa State College.
John A. Moore, Assistant Professor of Zoology, Barnard College.
Jean Brachet, Professor of Experimental Morphology, University Brussels.

III. RESEARCH ASSISTANT

Marjorie Hopkins Fox, University of California.

IV. LABORATORY ASSISTANTS

Catherine Henley, The Johns Hopkins University.
Alice H. Ferguson.

PHYSIOLOGY

I. CONSULTANTS

William R. Amberson, Professor of Physiology, University of Maryland, School of Medicine.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.

II. INSTRUCTORS

Arthur K. Parpart, Associate Professor of Biology, Princeton Universtiy, in charge of course.
E. S. Guzman Barron, Associate Professor of Biochemistry, The University of Chicago.
Rudolph T. Kempton, Professor of Zoology, Vasser College (absent in 1946).
M. J. Kopac, Assistant Professor, New York University.
George Wald, Associate Professor of Biology, Harvard University.
Dorothy Wrinch, Lecturer, Smith College.

BOTANY

I. CONSULTANTS

S. C. Brooks, Professor of Zoology, University of California.
P. R. Burkholder, Eaton Professor of Botany, Yale University.
W. R. Taylor, University of Michigan.

II. INSTRUCTORS

Maxwell S. Doty, Instructor of Botany, Northwestern University. In Charge of Course.
Hannah Croasdale, Dartmouth College.
Isabella Abbott, University of California.

III. RESEARCH ASSISTANTS

R. D. Northcraft, Massachusetts State College.
R. D. Wood, Northwestern University.

IV. Laboratory Assistant

Edwin T. Moul, University of Pennsylvania.

EXPERIMENTAL RADIOLOGY

G. Failla, College of Physicians and Surgeons, Columbia University.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian
Deborah Lawrence, Assistant Librarian
Margaret P. McInnis
Mary A. Rohan
Jean Goodfellow

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. Graham
Dorothy LeFevre

CHEMICAL DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

James McInnis, Manager
John S. Rankin, Naturalist
Ruth Crowell
Marcia McLaughlin
M. B. Gray
A. M. Hilton
W. E. Kahler
W. S. Landers
G. Lehy
R. E. Tonks
F. N. Whitman

GENERAL OFFICE

F. M. MacNaught, Business Manager
Homer P. Smith, Assistant Business Manager
Polly L. Crowell
Mrs. Lila S. Myers

GENERAL MAINTENANCE

T. E. Larkin, Superintendent
Robert Adams
R. W. Kahler
Geo. Kahler
G. T. Nickelson, Jr.
A. J. Pierce
T. E. Tawell

THE GEORGE M. GRAY MUSEUM

3. Investigators and Students

Independent Investigators, 1947

Abbott, Isabella A., Graduate Student, University of California.
Abelson, Philip H., Chairman Bio Physics Section, Carnegie Inst. of Washington.
Allen, M. Jean, Instructor in Biology, Mather College, Western Reserve University.

Anderson, Rubert S., Assistant Professor of Physiology, University of Maryland Medical School.
Armstrong, Philip B., Professor of Anatomy, College of Medicine, Syracuse University.
Atlas, Meyer, Assistant Professor of Biology, Yeshiva University.
Baez, Silvio, Research Fellow, Cornell Medical College.
Ball, Eric G., Professor of Biological Chemistry, Harvard Medical School.
Barron, E. S. Guzman, Associate Professor of Biochemistry, The University of Chicago.
Bartlett, James H., Professor of Theoretical Physics, University of Illinois.
Barton, Eleanor, Assistant Professor of Zoology, N. J. College for Women, Rutgers University.
Berger, Charles A., Director, Biological Laboratory, Fordham University.
Bliss, Alfred F., Assistant Professor of Physiology, Tufts College Medical School.
Blum, Harold F., Guggenheim Fellow, National Cancer Institute.
Boell, E. J., Professor of Biology, Yale University.
Bonner, John T., Junior Fellow, Harvard University.
Brachet, Jean L. A., Visiting Professor, University of Pennsylvania.
Bridgman, Josephine, Associate Professor of Biology, Limestone College.
Bronk, Detlev W., Professor of Biophysics and Director, Johnson Foundation, University of Pennsylvania.
Brown, Frank A., Jr., Professor of Zoology, Northwestern University.
Brownell, Katherine A., Instructor, Ohio State University.
Brust, Manfred, Graduate Student, University of Chicago.
Budington, Robert A., Professor of Zoology Emeritus, Oberlin College.
Buno, Washington, Professor and Director Department of Histology, School of Medicine, Montevideo.
Burbanck, W. D., Professor of Biology, Drury College.
Burger, Andre, Visitor at Harvard University.
Chambers, Francis W., Jr., Lt. Com. U. S. Navy through Columbia University.
Chambers, Robert, Research Professor of Biology, New York University.
Chase, Aurin M., Assistant Professor of Biology, Princeton University.
Cheney, Ralph H., Associate Professor of Biology, Brooklyn College.
Chrystall, Frieda L., Teacher of Biology, New York City Public High School.
Claff, C. Lloyd, Research Fellow in Surgery, Harvard Medical School.
Clark, A. M., Instructor in Biology, University of Delaware.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania School of Medicine.
Clark, L. B., Professor of Biology and Chairman of Department, Union College.
Clement, A. C., Professor of Biology, College of Charleston.
Clowes, G. H. A., Research Director Emeritus, Eli Lilly & Company.
Cole, Kenneth S., Professor of Biophysics, University of Chicago.
Colwin, Arthur L., Assistant Professor of Biology, Queens College.
Conklin, E. G., Professor Emeritus of Biology, Princeton University.
Cooper, Kenneth W., Associate Professor of Biology, Princeton University.
Copley, A. L., Research Associate, N. Y. U. Washington Square College.
Copeland, D. E., Assistant Professor of Zoology, Brown University.
Cornman, Ivor, Research Fellow, Sloan-Kettering Institute.
Costello, Donald P., Professor of Zoology, University of North Carolina.
Croadale, Hannah T., Associate in Zoology, Dartmouth College.
Crowell, Sears, Associate Professor of Zoology, Miami University.
Curtis, Winston C., Professor Emeritus of Zoology, University of Missouri.
Dan, Jean C., Independent Investigator, Misaki Marine Biological Station, Japan.
Doty, Maxwell S., Assistant Professor of Botany, Northwestern University.
Dumm, Mary E., Instructor in Chemistry, New York University Medical College.
Duryee, William R., Guest Investigator, Carnegie Institute of Washington.
Edgerley, Robert H., Assistant Professor of Zoology, University of Alabama.
Eichel, Bertram, Teaching Research Fellow, N. Y. U. College of Dentistry.
Evans, Titus C., Assistant Professor of Radiobiology, College of Physicians and Surgeons.
Failla, G., Professor of Radiology, College of Physicians and Surgeons.
Figge, Frank H. J., Associate Professor of Anatomy, University of Maryland School of Medicine.

Fisher, Harvey F., Western Reserve University.
Froehlich, Alfred, Associate, May Institute for Medical Research.
Gabriel, Mordecai L., Instructor of Biology, Brooklyn College.
Gilman, Lauren C., Assistant Professor of Zoology, University of South Dakota.
Glaser, Otto, Professor of Biology, Amherst College.
Goodchild, C. G., Professor of Biology, Missouri State College.
Goodrich, H. B., Professor of Biology, Wesleyan University.
Gorbman, Aubrey, Assistant Professor of Zoology, Columbia University.
Gould, Harley N., Professor of Biology, Newcomb College.
Grosch, Daniel S., Assistant Professor, University of North Carolina.
Grundfest, Harry, Research Associate in Neurology, Columbia University Medical School.
Hall, Thomas S., Associate Professor of Zoology, Washington University.
Hamilton, Howard L., Assistant Professor of Zoology, Iowa State College.
Hartman, Frank A., Professor of Physiology, Ohio State University.
Harvey, Ethel Browne, Research Biology Department, Princeton University.
Harvey, E. Newton, Professor of Physiology, Princeton University.
Heidenthal, Gertrude, Assistant Professor of Biology, Russell Sage College.
Heilbrunn, L. V., Professor of Zoology, University of Pennsylvania.
Hibbard, Hope, Professor of Zoology, Oberlin College.
Hickson, Anna Keltch, Research Chemist, Eli Lilly & Company.
Hinton, Taylor, Instructor, Amherst College.
Hopkins, Hoyt S., Associate Professor of Physiology, N. Y. U. College of Dentistry.
Hsiao, Sidney C., Visitor and Seessel Fellow, Yale University.
Hutchings, Lois M., Instructor, N. Y. U. Washington Square College.
Ifft, John D., Assistant Professor of Biology, Simmons College.
Jablons, Benjamin, Associate Clinical Professor, New York Medical College.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania.
Jenkins, George B., Emeritus Professor of Anatomy, George Washington University.
Jerome, Sister Francis, Professor of Biology, Ohio State University.
Karush, Fred, Research Associate, N. Y. U. Medical School.
Kemp, Margaret, Associate Professor of Botany, Smith College.
Kempton, Rudolph T., Professor of Zoology, Vassar College.
Kisch, Bruno, Professor at Yeshiva University.
Kitchen, I. C., Research Fellow, Princeton University.
Klotz, Irving M., Assistant Professor of Chemistry, Northwestern University.
Kopac, M. J., Associate Professor of Biology, N. Y. U. Washington Square College.
Krahl, M. E., Assistant Professor of Pharmacology, Washington University.
Kreezer, George L., Guggenheim Fellow, Princeton University.
Krugelis, Edith J., Research Associate, University of Pennsylvania.
Kuffler, Stephen W., Assistant Professor of Physiological Optics, Johns Hopkins Medical School.
Lavin, George I., in charge of Spectroscopic Laboratory, Rockefeller Institute for Medical Research.
Lazarow, Arnold, Assistant Professor of Anatomy, Western Reserve University.
LeFevre, Paul G., Instructor in Physiology, University of Vermont.
Libet, Benjamin, Instructor in Physiology, University of Chicago.
Liu, Chien-Kang, Graduate Student, McGill University.
Lillie, Ralph S., Professor Emeritus of Physiology, University of Chicago.
Lochhead, John H., Assistant Professor of Zoology, University of Vermont.
Lynn, W. Gardner, Associate Professor, The Catholic University of America.
MacDougall, Mary S., Professor of Zoology, Agnes Scott College.
Marmont, George, Assistant Professor of Biophysics, University of Chicago.
Marinelli, L., Physicist, Memorial Hospital.
Marshak, Alfred, Research Associate, New York University Medical College.
Marsland, Douglas A., Associate Professor of Biology, N. Y. U. Washington Square College.
Mavor, James W., Research Professor of Biology, Union College.
Mazia, Daniel, Associate Professor of Zoology, University of Missouri.
McDonald, Sister Elizabeth Seton, Professor of Biology, College of Mt. St. Joseph.

Memhard, Allen R., Riverside, Connecticut.
Metz, Charles B., Assistant Professor of Zoology, Yale University.
Metz, C. W., Chairman, Department of Zoology, University of Pennsylvania.
Miller, James A., Chairman, Department of Anatomy, Emory University.
Moore, John A., Assistant Professor of Zoology, Barnard College.
Moul, Edwin T., Assistant Instructor of Botany, University of Pennsylvania.
Nabrit, S. M., Professor of Biology, Atlanta University and Moreland College.
Nachmansohn, David, Research Associate in Neurology College of Physicians and Surgeons.
Nie, Dashu, Research Fellow, Institute of Zoology, Academia Sinica, China.
Northrop, John H., Member of the Institute, Rockefeller Institute, Princeton.
O'Brien, John A., Jr., Assistant Professor of Biology, Catholic University of America.
Olmsted, Frederick, Member Research Staff, Cleveland Clinic.
Osterhout, W. J. V., Member Emeritus, Rockefeller Institute for Medical Research.
Pappenheimer, A. M., Associate Professor of Bacteriology, New York University.
Parmenter, Charles L., Professor of Zoology, University of Pennsylvania.
Parpart, Arthur K., Professor of Biology, Princeton University.
Patt, Donald I., Instructor in Biology, Middlebury College.
Pequegnat, Willis E., Assistant Professor of Zoology, Pomona College.
Pierce, Madelene E., Associate Professor, Vassar College.
Plough, Harold H., Professor of Biology, Amherst College.
Prosser, C. Ladd, Associate Professor of Zoology, University of Illinois.
Reid, W. Malcolm, Associate Professor and Department Head of Biology, Monmouth College.
Robbie, Wilbur A., Research Assistant Professor, State University of Iowa.
Rogick, Mary D., Professor of Biology, College of New Rochelle.
Roofe, Paul G., Professor of Anatomy, University of Kansas.
Roth, Alexander, Research Assistant, University of Kansas.
Rudzinski, Anna Maria, Research Worker, Washington Square College.
Rugh, Roberts, Associate Professor of Biology, N. Y. U. Washington Square College.
Rulon, Olin, Assistant Professor of Biology, Wayne University.
Schmidt, Gerhart, Research Fellow, Tufts Medical School.
Schneyer, Leon H., Instructor, New York University Dental College.
Scott, Allan, Associate Professor of Biology, Union College.
Shanes, Abraham M., Assistant Professor of Physiology, New York University College of Dentistry.
Shapiro, Herbert, Physiologist, National Institute of Health.
Sherman, Frederick G., Instructor of Biology, Brown University.
Sichel, F., Professor of Physiology, University of Vermont.
Slifer, Eleanor H., Assistant Professor of Zoology, State University of Iowa.
Smith, Sydney, University Lecturer, Cambridge University, England.
Stewart, Dorothy R., Assistant Professor of Anatomy, Western Reserve Medical School.
Speidel, Carl C., Professor of Anatomy, University of Virginia.
Steinbach, H. B., Professor of Zoology, University of Minnesota.
Stokey, Alma G., Professor Emeritus, Mount Holyoke College.
Stoudt, Harry N., Instructor in Biology, Temple University.
Straus, William L., Associate Professor of Anatomy, Johns Hopkins University.
Stunkard, Horace W., Professor of Biology, New York University.
Szent-Gyorgyi, Albert, Professor of Biochemistry, Budapest University, Budapest.
Taylor, Wm. Randolph, Professor of Botany, University of Michigan.
Townsend, Grace G., Professor of Biological Science, Cincinnati College of Pharmacy.
Tracy, Henry, Professor of Anatomy, University of Kansas.
Treitel, Otto, Research Associate, Botanical Laboratory, University of Pennsylvania.
Villee, Claude A., Instructor in Biological Chemistry, Harvard Medical School.
Wainio, Walter W., Assistant Professor, New York University College of Dentistry.
Wald, George, Associate Professor, Harvard University.
Ward, Helen L., Instructor in Zoology, University of Tennessee.
Warner, Robert C., Assistant Professor of Chemistry, New York University College of Medicine.
Waterman, Talbot, Instructor,Yale University.

Watterson, Ray L., Assistant Professor of Zoology, University of Chicago.
Whiting, Anna R., Guest Investigator, University of Pennsylvania.
Whiting, P. W., Professor of Zoology (Genetics), University of Pennsylvania.
Wichterman, Ralph, Associate Professor of Biology, Temple University.
Wilber, Charles G., Assistant Professor of Physiology, Fordham University.
Wilde, C. E., Jr., Instructor of Biology, Princeton University.
Winsor, Charles P., Assistant Professor, School of Hygiene, Johns Hopkins University.
Witkus, Eleanor R., Instructor in Botany and Cytology, Fordham University.
Wrinch, Dorothy, Lecturer, Smith College.
Yntema, Chester L., Associate Professor of Anatomy, Syracuse University Medical College.
Zinn, Donald J., Instructor in Zoology, Rhode Island State College.
Zorzoli, Anita, Instructor in Physiology and Biochemistry, Washington University.
Zweifach, Benjamin W., Assistant Professor of Physiology, Cornell Medical College.

Beginning Investigators, 1947

Alscher, Ruth P., Instructor in Biology, Manhattanville College.
Allen, Mary Belle, Research Fellow, Washington University.
Berg, George G., Graduate Student, Columbia University.
Blumenthal, Gertrude, University of Pennsylvania.
Bronfenbrenner, Alice, Medical Student, Washington University Medical School.
Crane, Robert K., Graduate Student, Harvard University.
Fahey, Elizabeth M., Boston University.
Ferguson, Alice, Graduate Assistant, Louisiana State University.
Fogerson, Virginia L., Assistant in Zoology, Vassar College.
Fontanella, M. A., Instructor in Comparative Anatomy, Fordham University.
Fowle, Ann M. C., Research Assistant, University of Toronto.
Gollub, Seymour, Student, University of Pennsylvania.
Green, James W., Graduate Student, Princeton University.
Herbruck, Bruce K., Student, Western Reserve University School of Medicine.
Kuntz, Eloise, Assistant in Biology, Brown University.
Lerner, Eleanor, Fellow in Zoology, Washington University.
Lovelace, Roberta, Teaching Fellow, University of North Carolina.
Lumb, Ethel Sue, Graduate Assistant in Zoology, Washington University.
Martin, Barbara A., Assistant in Zoology, Barnard College.
McLean, Dorothy J., Demonstrator, University of Toronto.
Nelson, Thomas Clifford, Graduate Assistant, Columbia University.
Nurnberg, Miriam, Graduate Student, New York University.
Pettengill, Olive S., Graduate Assistant in Physiology, Brown University.
Ransmeier, Robert E., Graduate Student, University of Chicago.
Recknagel, Richard O., Student, University of Pennsylvania.
Schlesinger, Arthur H., Research Fellow, Northwestern University.
Shaver, John R., Instructor in Zoology, University of Pennsylvania.
Sirot, Gustave, Western Reserve University.
Taylor, Babette, Graduate Student, University of Minnesota.
Tietze, F., Research Fellow, Northwestern University.
Van Hoesen, Drusilla, Graduate Student, University of Pennsylvania.
Weiner, Milton H., Student, Western Reserve University.
Weinstein, H. G., Research Assistant, University of Illinois.
Wilson, Jean R., Graduate Student, University of Pennsylvania.
Wilson, Walter L., Assistant Instructor in Zoology, University of Pennsylvania.
Wittenberg, Jonathan, Student, Columbia University.

Research Assistants, 1947

Abramsky, Tess, Research Assistant, Rockefeller Institute for Medical Research.
Benson, Eleanore, Research Assistant, University of Pennsylvania.
Berman, Jack H., Graduate Student, Western Reserve University.
Berman, Ruth, Student and Research Assistant, University of Pennsylvania.
Carlson, Francis D., Research Assistant, Johnson Foundation, University of Pennsylvania.

Clippinger, F. W., Student, Drury College.
Connelly, C. M., Graduate Student, Johnson Foundation, University of Pennsylvania.
Cooper, Octavia, Research Assistant, Harvard Medical School.
Cooperstein, Sherwin, Student, New York University College of Dentistry.
Danufsky, Philip, Research Assistant, University of Pennsylvania.
Dey, Thomas E., Research Technician, Princeton University.
Dietz, Alma, Student, University of Michigan.
Estable, Jose J., Professor of Pharmacology, University of Montevideo, Uruguay.
Ferreira, Hiss M., Fellow in Biophysics, University of Chicago.
Foley, Mary T., Research Assistant, Yale University.
Fox, Marjorie Hopkins, Instructor, San Francisco Junior College.
Gold, Marcia, Research Assistant, University of Chicago.
Hendee, Edelmira D., Research Assistant, New York University Medical School.
Henley, Catherine, Research Assistant, University of North Carolina.
Hirschhorn, Henry A., Student, New York University.
Hoare, Catherine V., Graduate Assistant in Bacteriology, Brown University.
Honegger, Carol M., Instructor, Temple University.
Jaffe, Lionel F., Harvard University.
Kemp, Grace, Graduate Assistant, Wesleyan University.
Metz, Delilah B., Research Associate in Medicine, Cornell Medical School.
Mezger, Liselotte, Washington University.
Mitchell, Constance, Research Assistant, University of Pennsylvania.
Neff, Robert J., Research Assistant, University of Missouri.
Norris, Karl H., Electronic Engineer, University of Chicago.
Northcraft, Richard D., Instructor, Rutgers University.
Portis, Richard A., Graduate Student, Western Reserve University.
Rall, Wilfrid, Fellow in Biophysics, University of Chicago.
Rieser, Peter, Research Assistant, University of Pennsylvania.
Rothenberg, M. A., Research Assistant in Neurology, Columbia University.
Sandeen, Muriel, Teaching Assistant, Northwestern University.
Seaman, Gerald R., Graduate Assistant, Fordham University.
Volkman, Alvin, Graduate, Union College.
Walters, C. Patricia, Research Assistant, Eli Lilly & Company.
Webb, Marguerite, Teaching Assistant, Northwestern University.
Weiss, Michael S., Research Assistant in Neurology, College of Physicians and Surgeons.
Wheeler, Charles B., Research Assistant, Anatomy Department, University of Kansas.
Willis, Marion, Research Assistant, University of Pennsylvania.
Wilson, Marie, Assistant in Zoology, Northwestern University.
Woodward, Arthur A., Jr., Instructor in Zoology, University of Pennsylvania.
Young, Marcia A., Technical Assistant, Ohio State University.

Library Readers, 1947

Berg, William E., Research Fellow in Medical Physics, University of California.
Bernheimer, Allan W., Assistant Professor of Bacteriology, New York University College of Medicine.
Bloch, Robert, Research Associate in Botany, Yale University.
Cantoni, G. L., Assistant Professor in Pharmacology, Long Island College of Medicine.
Clifford, Sister Adele, Teacher, College of Mount St. Joseph.
Deane, Helen W., Instructor, Harvard Medical School.
Fries, E. F. B., Assistant Professor of Biology, City College of New York.
Gates, R. Ruggles, Research Fellow in Biology, Harvard University.
Grant, Madeleine, Member Teaching Faculty, Sarah Lawrence College.
Gudernatsch, Frederick, Visiting Professor, New York University.
Gurewich, Vladimir, Assistant Visiting Physician, Bellevue Hospital.
Harrison, John W., Student, Medical School, University of Pennsylvania.
Hobson, Lawrence B., Assistant. Resident, New York Hospital.

Joftes, David L., Assistant in Biology, Tufts College.
Jonassen, Hans B., Assistant Professor of Chemistry, Tulane University.
Lange, Mathilde M., Professor of Zoology, Wheaton College.
Lederberg, Joshua, Fellow, Jane Coffin Childs Fund, Yale University.
Levitt, Leo C., Graduate Student in Physics, Princeton University.
Levey, Stanley, Instructor in Biochemistry, Wayne University College of Medicine.
Loewi, Otto, Research Professor of Pharmacology, New York University College of Medicine.
Meyershof, Otto, Research Professor of Biochemistry, University of Pennsylvania.
Mouton, Robert F., Head of Mission, Belgium.
Oster, Robert H., Associate Professor of Physiology, University of Maryland School of Medicine.
Pick, Joseph, Associate Professor of Anatomy, New York University College of Medicine.
Price, Bronson, Analyst, U. S. Public Health Service.
Rose, S. Meryl, Assistant Professor, Smith College.
Ryan, Francis J., Assistant Professor of Zoology, Columbia University.
Schneider, Lillian K., Research Assistant in Microbiology, Columbia University.
Schwartzman, Gregory, Head of Department of Bacteriology, Mt. Sinai Hospital.
Siegel, Benjamin, Associate in Laboratory of Electron Microscopy, Polytechnic Institute.
Spratt, Nelson T., Jr., Assistant Professor of Biology, Johns Hopkins University.
Taussky, Hertha H., Research Associate, Cornell University Medical College.
VonSallman, Ludwig, Associate Professor, College of Physicians and Surgeons.
Wagman, Irving H., Associate in Physiology, Jefferson Medical College.
Watts, Nellie P., Pharmacologist, Abbott Laboratories.
Zawadzki, Bronislaw, Fellow, College of Physicians and Surgeons.

Students, 1947

BOTANY

Boyle, E. Marie, Science Teacher, Baldwin School.
Cadoret, Remi, Student, Harvard College.
Coyle, Elizabeth E., Assistant Professor of Biology, College of Wooster.
Dearden, Elizabeth R., Research Assistant, University of Toronto.
Dippell, Ruth D., Research Associate, Indiana University.
Dworkin, Zelman Z., Instructor, University of Connecticut.
Erskine, David S., Acadia University.
Ferguson, Edward L., Undergraduate, Wesleyan University.
Gage, Marilyn A., Student, Pennsylvania College for Women.
Grimm, Madelon R., Research Assistant in Bacteriology, Amherst College.
Holmes, Robert W., Student, Haverford College.
Hulburt, Edward M., Graduate Student, University of Michigan.
Hyde, Beal B., Student, Harvard University.
Lawson, Dorothy L., Wellesley College.
Spiegel, Leonard E., Drew University.
Wood, Richard D., Northwestern University.
Wood, Urda K., Northwestern University.

EMBRYOLOGY

Bauer, Mark H., Graduate Student, Princeton University.
Blair, Charles B., Graduate Student, Instructor, University of North Carolina.
Bolton, Ellis T., Graduate Assistant, Rutgers University.
Buchanan, Douglas, Assistant in Biochemistry, Dartmouth Medical School.
Calvert, Julie N., Demonstrator in Biology, Bryn Mawr College.
Chmielowski, Adam A., Graduate Assistant, Marquette University.
Cloud, Preston E., Jr., Assistant Professor of Geology, Harvard University.
Cortelyou, Rev. J. R., Student, Northwestern University.

Dickason, Mary E., Smith College.
Edwards. John P., Graduate, Drury College.
Gomberg, Charles, Student, McGill University.
Gregg, James H., Graduate Student, Princeton University.
Hill, Henrietta J., Dickinson College.
Hinchey, M. Catherine, Instructor in Biology, Temple University.
Holtzer, Howard, Student, University of Chicago.
Hopkins, Amos L., Harvard College.
Isaac, Isaac B., Wesleyan University.
Klau, Helene H., Graduate Assistant, University of Oklahoma.
Leone, Charles A., Instructor, Rutgers University.
Magdebarger, Alice E., Student, Goucher College.
Maxon, Marion G., Graduate Assistant, Claremont Graduate School.
Nelson, Betty G., Graduate Student, Johns Hopkins University.
Odum, Howard T., Graduate Student, University of North Carolina.
Padykula, Helen A., Graduate Assistant, Mt. Holyoke College.
Riggs, Austin F., Student, Harvard College.
Robbins, Marilyn, Student, Stanford University.
Rosenbloom, Libby, Laboratory Assistant, University of Michigan.
Russo, Evelyn E., Rosemont College.
Shrader, Ruth E., Graduate Student, Yale University Medical School.
Stolack, Richard B., Graduate Assistant, University of North Carolina.
Weinstein, Hyman G., Research Assistant, University of Chicago.
Wenger, Byron S., Graduate Assistant, Washington University.
Zuckerkandl, Emil, University of Illinois.

PHYSIOLOGY

Ardao, Maria I., Assistant Professor of Chemistry, Montevideo, Uruguay.
Bateman, Margaret M., Graduate Student, Washington University.
Bicks, Richard O., Long Island College of Medicine.
Bloch, Samuel W., Hammond, Louisiana.
Cosgrove, William B., Graduate Assistant, New York University.
Das, S. M., Government Scholar, Government of India.
Edelberg, Robert E., Graduate Student, University of Pennsylvania.
Fiala, Silvio, Charles University, Prague.
Friedman, Florence L., Teaching Assistant, Washington University.
Gourley, D. R. H., Research Assistant, University of Toronto.
Hamilton, James D., Fellow, Department of Medical Research, University of Western Ontario.
Hanke, Harriett, Teaching Fellow in Biology, New York University.
Irving, Jack Howard, Graduate Student, Princeton University.
Kaupe, Walter, Massachusetts Institute of Technology.
Kellogg, Ralph H., Teaching Fellow in Physiology, Harvard Medical School.
Layton, Laurence L., Assistant Professor, Johns Hopkins University.
Love, Warner E., Graduate Student, University of Pennsylvania.
McCann, William P., Cornell University Medical College.
Moskovic, Samuel, Graduate School, New York University.
Nelson, Leonard, Graduate Teaching Assistant, Washington University.
Paulsen, Elizabeth C., Instructor in Zoology, University of Vermont.
Perlman, Preston L., Research Fellow, Cornell University.
Ransmier, Robert E., Graduate Student, University of Chicago.
Steele, Alloys C., Research Fellow, University of Toronto.
Stekler, Burton, Student, New York University Medical School.
Taylor, Robert E., Fellow in Physiology, Strong Memorial Hospital.

INVERTEBRATE ZOOLOGY

Allen, John M., Drury College.
Alscher, Ruth P., Instructor in Biology, Manhattanville College.
Anderton, Laura G., Laboratory Assistant, Brown University.
Bauer, Edward Charles, Undergraduate Assistant, University of Connecticut.
Boss, Willis R., University of Iowa.
Boyer, George F., Graduate Student, University of Illinois.
Bragg, Nana I., Student, Oberlin College.
Bucklin, Donald H., Graduate Assistant, Amherst College.
Bull, Alice L., Laboratory Assistant, Mt. Holyoke College.
Burger, Andre, Harvard University.
Chamberlin, J. Lockwood, Tufts College.
Cloud, Preston E., Assistant Professor, Harvard University.
Cole, Gerald A., Laboratory Assistant, University of Minnesota.
Corliss, Clark E., Graduate Student, University of Massachusetts.
Daniel, Edwin E., Student, Johns Hopkins University.
Ehrlich, Miriam, Graduate Student, Yale University.
Epstein, Howard E., Assistant, Department of Comparative Anatomy, Cornell University.
Evans, Jeanne F., Student, University of Pennsylvania.
Flynn, Joyce M., Newton, Massachusetts.
Ford, Benjamin P., Princeton University.
Ford, Donald H., Laboratory Assistant, Wesleyan University.
Ford, Priscilla W., Wellesley College.
Fuller, Forst D., Instructor in Zoology, Purdue University.
Glockler, Annabel, Western Maryland College.
Goodsmith, Willoughby, Teaching Assistant and Student, Amherst College.
Hay, Elizabeth D., Undergraduate, Smith College.
Henoch, Stephanie D., Graduate Assistant, Indiana University.
Howard, Robert S., University of Chicago.
Hutchings, Lois M., Instructor, N. Y. U. Washington Square College.
Kamner, Sandra L., Goucher College.
Keller, Mildred E., Randolph-Macon Woman's College.
Mandlowitz, Samuel, Tulane University.
McCullough, Kirk W., Instructor, Washington and Jefferson College.
McElligott, Jane K., Graduate Student, Fordham University.
McGuire, Irene E., Fordham University.
McWhinnie, Mary A., Instructor in Zoology, DePaul University.
Meltzer, Jay, Student, Princeton University.
Odum, Howard T., Graduate Student, University of North Carolina.
Perna, Vincent, Student, Wesleyan University.
Pronko, Robert C., Student, Drury College.
Radford, Betty J., Assistant in Biology Department, Agnes Scott College.
Sause, Gladys E., Western Maryland College.
Springer, Eleanor V., Vassar College.
Stay, Barbara A., Student, Vassar College.
Steeves, Taylor A., East Weymouth, University of Massachusetts.
Stolack, Richard B., Graduate Student, University of North Carolina.
Susca, Louis, Graduate Student, Fordham University.
Thayer, Philip S., Amherst College.
Trent, Jane, Assistant, Wesleyan University.
Wenger, Byron S., Graduate Assistant, Washington University.
Winn, Hudson S., Graduate Teaching Assistant, Northwestern University.
Winston, Paul W., Student, University of Massachusetts.
Withrow, Joanna E. F., Student, Oberlin College.
Wolf, Doris E., Graduate Student, University of Minnesota.
Wong, An Chi, Wellesley College.

4. Tabular View of Attendance

	1943	*1944*	*1945*	*1946*	*1947*
INVESTIGATORS—Total	160	193	212	267	299
Independent	89	112	138	175	182
Under instruction	19	11	10	29	36
Library readers	35	50	38	38	36
Research assistants	17	20	26	25	45
STUDENTS—Total	68	75	96	122	131
Zoology	47	37	55	57	55
Embryology	13	23	23	30	33
Physiology	8	10	13	26	26
Botany	—	5	5	9	17
TOTAL ATTENDANCE	228	276	308	389	430
Less persons registered as both students and investigators	6	1			2
	222	275			428
INSTITUTIONS REPRESENTED—TOTAL	116	106	124	141	
By investigators	70	74	100	102	114
By students	41	41	49	56	56
SCHOOLS AND ACADEMIES REPRESENTED					
By investigators	2	1	2	2	1
By students	1	2	2	—	1
FOREIGN INSTITUTIONS REPRESENTED					
By investigators	2	2	1	7	7
By students	—	3	—	5	3

5. Subscribing and Cooperating Institutions, 1947

Cooperating Institutions

American Philosophical Society (Penrose Fund)
Amherst College
Brooklyn College
Brown University
Bryn Mawr College
The Catholic University of America
College of Mt. St. Joseph on the Ohio
Columbia University
Cornell University
Duke University
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Johns Hopkins Medical School
Johns Hopkins University
Johnson Foundation
Eli Lilly & Company
Macy Foundation
Massachusetts Institute of Technology
Memorial Hospital
Miami University
New York University
New York University College of Medicine
New York University School of Dentistry
New York University Washington Square College
Oberlin College
Ohio State University
Pomona College
Princeton University
Rockefeller Institute of Medical Research
State University of Iowa
Syracuse University Medical School
Temple University
Tufts College
Union College
University of Chicago
University of Illinois
University of Kansas
University of Maryland Medical School
University of Missouri
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Rochester
University of Toronto
University of Vermont
University of Virginia
Vassar College
Washington University
Wayne University
Wellesley College
Wesleyan University
Western Reserve Medical School
Wilson College
Woods Hole Oceanographic Institution
Yale University

Subscribing Institutions

Atlanta University, Moreland College
Boston Dispensary
Carnegie Inst. of Washington
College of Charleston
College of Physicians and Surgeons
Indiana University
Mount Sinai Hospital
National Cancer Institute
North Carolina State College of Agriculture & Engineering
Pennsylvania College for Women
Rockefeller Foundation
Russell Sage College
Rutgers University
Simmons College
Smith College
St. John's College
University of Delaware
University of Massachusetts
University of Michigan
University of Minnesota
University of Western Ontario

6. The Friday Evening Lectures, 1947

June 27
Dr. Gottfried Fraenkel .... "Nutritional Research with Insects."

July 3
Dr. Jean Brachet .... "Metabolism of Nucleic Acids in Embryonic Development."

July 11
Prof. E. L. Tatum .... "Mutation in Microorganisms."

July 25
Dr. F. O. Schmitt .... "Studies of the Ultra Structure of Connective Tissue Constituents."

August 1
Dr. Philip H. Abelson .... "Tracer Isotopes in Biology."

August 8
Prof. Alfred S. Romer .... In celebration of the 74th Anniversary of the founding of the Agassiz Laboratory at Penikese.

August 15
Dr. John A. Moore .... "Early Development of Amphibian Hybrids."

August 22
Dr. Daniel Mazia .... "The Molecular Structure of Chromosomes."

Other Lectures

July 24
Dr. Paul S. Galtsoff .... "The Bikini Atom Bomb Test."

July 30
Dr. K. J. Heinicke .... "Recent Developments in Microscopy."

August 13
Dr. Oscar W. Richards .... "Phase Microscopy, with Special Reference to Biology."

August 18
Dr. H. J. Abraham .... U. S. State Dept. Associate Director of Relations with UNESCO.

August 27
Dr. Albert Szent-Gyorgyi .... "Muscular Contraction."

August 28
Prof. Louis van den Berghe .... "National Parks and Scientific Research in the Congo."

7. SEMINARS, 1947

July 15	ANNA R. WHITING	"Androgenetic Males from Eggs X-rayed with Dose Many Times Lethal."
	T. S. HALL AND FLORENCE MOOG	"Effects of Sodium Azide in Solution upon the Rate of Amphibian Development."
	C. A. VILLEE AND H. B. BISSELL	"Nucleic Acids and Nucleotides as Growth Factors in Drosophila."
July 22	IVOR CORNMAN	"The Effects of Podophyllin on the Maturation and Cleavage of the Starfish Egg."
	C. G. WILBER	"The Synthesis of Lipids from Protein in Colpidium Campylum."
	W. W. WAINIO, S. COOPERSTEIN, S. KOLLEN AND B. EICHEL	"The Preparation of a Soluble Cytochrome Oxidase."
July 29	OTTO MYERHOFF AND JEAN R. WILSON	"Glycolysis of Glucose, Fructose, and Hexosephosphates in Tumor and Brain Extracts."
	B. LIBET	"Relatively Steady Potentials and Brain Activity."
	J. T. BONNER	"Morphogenetic Movement in the Slime Molds."
August 5	EDITH J. KRUGELIS	"Alkalin Phosphatase Localization in Early Embryonic Development."
	J. R. SHAVER	"Experimental Study of the 'Second Factor' in Artificial Parthenogenesis in Frog Eggs."
	ROBERT BLOCH	"Irreversible Differentiation in Certain Plant Cell Lineages."
August 12	DOROTHY WRINCH	"Biological Specificity and Biological Morphology."
	TAYLOR HINTON	"Factors Influencing the Expression of 'Position Effects.'"
	D. E. COPELAND	"The Cytological Basis of Salt Excretion from the Gills of Fundulus heteroclitus."
August 19	P. W. WHITING	"Spermatogenesis in Sphecoid Wasps."
	ETHEL BROWNE HARVEY	"Bermuda Sea Urchins and Their Eggs."
	PAUL S. GALTSOFF	"The Red Tide along the Gulf Coast and Florida."

8. MEMBERS OF THE CORPORATION, 1947

1. LIFE MEMBERS

*ALLIS, MR. E. J., JR., Palais Carnoles, Menton, France.
BECKWITH, DR. CORA J., Vassar College, Poughkeepsie, New York.
BILLINGS, MR. R. C., 66 Franklin Street, Boston, Massachusetts.
CALVERT, DR. PHILIP P., University of Pennsylvania, Philadelphia, Pennsylvania.
COLE, DR. LEON J., College of Agriculture, Madison, Wisconsin.

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* Deceased.

Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Cowdry, Dr. E. V., Washington University, St. Louis, Missouri.
Jackson, Mr. Chas. C., 24 Congress Street, Boston, Massachusetts.
Jackson, Miss M. C., 88 Marlboro Street, Boston, Massachusetts.
King, Mr. Chas. A.
Kingsbury, Prof. B. F., Cornell University, Ithaca, New York.
Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Means, Dr. J. H., 15 Chestnut Street, Boston, Massachusetts.
Moore, Dr. George T., Missouri Botanical Gardens, St. Louis, Missouri.
Moore, Dr. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgan, Mrs. T. H., Pasadena, California.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
Scott, Dr. Ernest L., Columbia University, New York City, New York.
Sears, Dr. Henry F., 86 Beacon Street, Boston, Massachusetts.
Shedd, Mr. E. A.
Strong, Dr. O. S., Columbia University, New York City, New York.
Waite, Prof. F. C., 144 Locust Street, Dover, New Hampshire.
Wallace, Louise B., 359 Lytton Avenue, Palo Alto, California.

2. Regular Members

Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Massachusetts.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
Adolph, Dr. Edward F., University of Rochester Medical School, Rochester, New York.
Albaum, Dr. Harry G., Biology Dept., Brooklyn College, Brooklyn, N. Y.
Albert, Dr. Alexander, Mayo Clinic, Rochester, Minnesota.
Allee, Dr. W. C., The University of Chicago, Chicago, Illinois.
Amberson, Dr. William R., Department of Physiology, University of Maryland, School of Medicine.
Anderson, Dr. Rubert S., University of Maryland School of Medicine, Department of Physiology, Baltimore, Maryland.
Anderson, Dr. T. F., University of Pennsylvania, Philadelphia, Pennsylvania.
Angerer, Prof. C. A., Department of Physiology, Ohio State College, Columbus, Ohio.
Armstrong, Dr. Philip B., College of Medicine, Syracuse University, Syracuse, New York.
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Knower, Mrs. Henry
Lillie, Mrs. Frank R.
Marvin, Mrs. A. H.
Mitchell, Mrs. James McC.
Mixter, Mrs. Jason
Moore, Mrs. William A.

Motley, Mrs. Thomas
Murphy, Dr. Walter J.
Newton, Miss Helen
Nims, Mrs. E. D.
Norman, Mr. and Mrs. Edward
Oppenheim-Errer, Dr. and Mrs. Paul
Park, Malcolm
Rentschler, Mr. and Mrs. George
Riggs, Mrs. Lawrason
Rudd, Mrs. H. W. Dwight
Saunders, Mrs. Lawrence
Spackman, Miss Emily S.
Steel, Richard
Stockard, Mrs. Charles R.
Strecher, Mrs.
Swope, Gerard
Tebbetts, Mr. and Mrs. Walter
Truslow, Mr. and Mrs. Arthur
Ward, Mr. and Mrs. Francis T.
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PROTOPLASMIC VISCOSITY CHANGES DURING MITOSIS IN THE EGG OF CHAETOPTERUS

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In spite of the enormous effort that has been spent in order to discover the basic cause of cancer, a disease primarily due to the initiation of cell division in cells which normally do not divide, there has been but little advance in the past twenty years in our understanding of the basic physiology of cell division. Of the three main theories which have been proposed to account for the initiation of cell division, only one survives and that has had but little test. The idea that cell division is caused by an increase in cell permeability can scarcely be held at the present time, for in marine egg cells the calcium ion is a potent agent for promoting mitosis (See Pasteels, 1941; Hollingsworth, 1941) and the calcium ion is well known for its effect in decreasing rather than increasing cell permeability. Secondly, at the present time it can hardly be maintained that an increase in respiration is the primary cause of initiation of cell division. When certain types of cells are incited to divide, the respiration does increase, but other types of cells show no such effect, and in still other cells the respiration decreases (Whitaker, 1931a, b, c; 1933a, b; compare also Holter and Zeuthen, 1944). Nor, on the basis of present evidence, can it be held that a particular respiratory system is involved; at any rate the attempt to argue that in the sea-urchin egg the cytochrome oxidase system is activated when the cell is incited to divide is rather an expression of wishful thinking than of careful experimentation (See Robbie, 1946). We are left then with the third of the three major theories, the view that the primary impetus to cell division is a mitotic gelation akin to the gelation which occurs generally in cells when they respond to stimulation. This colloidal theory of cell division is discussed at some length in the second edition of Heilbrunn's Outline of General Physiology (1943, see Chapt. 42).

One of the reasons for believing in the colloidal theory is that in those cells in which viscosity studies have been made, the appearance of the mitotic spindle appears to be preceded by a very definite gelation of the cytoplasm. Actually, however, only a few types of cells have been studied, and if this point is to be firmly established, we should have additional information for other types of cells.

We were led to a study of the egg of the annelid Chaetopterus pergamentaceus Cuvier, because this worm is found in suitable numbers at Woods Hole and can be obtained for study, and also because the egg represents a type similar to Cumingia in that fertilization occurs at the time of the first maturation spindle. Unfortunately, at the present time Cumingia is very rare at Woods Hole and therefore can not be used for experimental work.

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Chaetopterus eggs can be obtained throughout the summer (Compare Mead, 1898). One female worm provides enough eggs for several experiments. When the eggs are shed into the sea water, they are in the germinal vesicle stage, but as soon as they enter the sea water, maturation begins and proceeds until the metaphase stage of the first maturation division is reached. Then, if the egg is fertilized, the maturation divisions continue and cleavage follows.

Obviously, the Chaetopterus egg is convenient for study. We plan to use it in various types of experimental work. We were interested therefore in knowing the normal cycle of viscosity change and how this cycle was related to the mitotic phenomena occurring between the time of fertilization and the first cleavage. Strangely enough, in spite of the great amount of cytological work on mitosis, there is no complete minute by minute time record of what can be seen in fixed and sectioned material during the progressive stages of mitosis in marine eggs.

METHODS

The sexes of Chaetopterus can be determined from the color of the gametes contained in the transparent parapodia. The eggs are yellow and the sperm are white. A few posterior segments of a worm were cut off and placed in a small stender dish containing about 20 ml. of sea water. The parapodia of these excised segments were cut open and as a result the eggs or the sperm, as the case might be, exuded into the sea water. Eggs were filtered through cheesecloth to remove the excess jelly and extraneous tissue and then were washed by decantation. The eggs are usually so abundant that only a few segments of the worm provide enough eggs for a single experiment. A sperm suspension was prepared by removing the parapodia and segments from the stender dish and then adding to the original 20 ml. another 10 ml. of sea water. Two or three drops of this suspension were used to fertilize one batch of eggs. A worm does not die following the removal of several segments and indeed the same worm may be used several times.

In all of our work, the eggs of only one female were used in any given series of experiments. The eggs were kept in a water bath maintained at a constant temperature of 21° C. In some experiments, the temperature varied slightly, but ordinarily the variation was not greater than two or three tenths of a degree. As soon as the eggs began to show indications of cleavage, counts were made as rapidly as possible in order to determine with reasonable precision the exact time at which 50 per cent of the eggs had divided. In these counts, all eggs in which a cleavage plane had begun to travel across the egg were regarded as cleaved. Actually, the passage of the cleavage plane through the egg takes an appreciable time, perhaps a minute or more, so that the cleavage times we recorded are somewhat less than the times would be if we considered as cleavage time the time at which the egg is completely divided. In making rapid counts on living eggs, it would scarcely be possible to use as a criterion of cleavage the complete division of the egg. At a temperature of 21° C. we found the average cleavage time to be 56 minutes.

Protoplasmic viscosity tests were made with an Emerson hand centrifuge. The handle of the centrifuge was turned once every two seconds. This represents a speed of 85 revolutions per second. The radius of turn was 8 cm. The centrifugal force was calculated to be 2325 times gravity. A few preliminary tests of protoplasmic viscosity during mitosis showed that the viscosity changes in the dividing Chaetopterus egg are not as pronounced as they are in the egg of Cumingia or in the egg of Arbacia. If tests are to be made at frequent intervals, observations must be made rapidly. This can introduce uncertainty. We were worried over the possibility that subjective impressions might creep in. Accordingly, we decided on the following procedure. For any given test, the centrifuge was turned by Mrs. Jean Wilson. As soon as the turning was completed, she passed the centrifuge tube to one of us as quickly as possible. The eggs were then removed from the tube to two microscope slides. Each of us had a microscope, and we observed the centrifuged eggs independently. In this way, we were able to make tests at one minute intervals and although the observations were necessarily very rapid, when we compared our results at the end of a series of tests, we found remarkably good agreement.

When a Chaetopterus egg is centrifuged, lighter (presumably fat) granules move to the centripetal pole, and heavier yolk granules move to the centrifugal pole. There is a cortical layer of granules which does not move at all. Details of the appearance of centrifuged eggs are given by Lillie (1906). When we observed the centrifuged eggs, we recorded everything that we could see. If the viscosity is relatively low, the yolk granules move more readily through the egg; the fat granules also move more readily. It is possible to observe a shift of the yolk granules before any movement of the fat granules can be noted. One can follow viscosity change either by considering the movement of heavy or light granules. In order to obtain definite quantitative values for viscosity, we chose as an endpoint the appearance of a definite accumulation of fat granules at the centripetal pole of the egg. The number of seconds required to give this accumulation was used in plotting a viscosity curve.

The eggs of Chaetopterus are not very transparent. We wanted to know exactly what was happening in the mitotic process during every minute between fertilization and cleavage. This necessitated preparation of sections. Eggs were fixed at one minute intervals either in Bouin's fluid or in Meves' fluid. We fixed four complete series of eggs, and all four series were imbedded in paraffin. Sections were made from only one series (Bouin), the others being kept in reserve. Sectioning and staining were done by Miss Drusilla Van Hoesen. Our sections are eight microns thick and they are stained with Heidenhain's hematoxylin.

Results

The viscosity changes in the protoplasm of the Chaetopterus egg during the period between fertilization and cleavage are shown in Figure 1. The viscosity figures represent relative values and they have no absolute basis other than that they represent the number of seconds of centrifugation necessary to arrive at the endpoint described in the previous section. In order to obtain the viscosity values, after some preliminary tests, we ran through 12 complete series, in each of which the eggs were centrifuged at minute intervals for a given length of time. The times of centrifuging in the various tests were 4, 5, 7, 9, 10, 11, 12, 13, 14, 15, 17, and 18 seconds.

Fertilization is followed by a drop in viscosity; then while the maturation divisions are proceeding, the viscosity remains constant. In one or two of our series, we did get some indication that during the course of the maturation divisions, there might be minor fluctuations in viscosity, but the weight of evidence is against any change whatsoever. This may seem strange, for in the egg of the clam Cumingia one of us had noted marked changes in viscosity during the maturation divisions (Heilbrunn, 1921). The difference between the egg of Chaetopterus and that of Cumingia is, however, easy to understand. In the relatively small egg of Cumingia, the maturation spindles occupy a rather large fraction of the egg volume. Thus Morris (1917) states that the first polar spindle "is large, and lies near the center of the egg. It might, in fact, be mistaken for a cleavage spindle in the metaphase, if it were not for the form of the chromosomes." Similarly, Jordan (1910) shows the first maturation spindle of Cumingia as a large structure extending through most of the egg; the distance between the centers of the centrospheres of this spindle is approximately half the diameter of the egg. On the other hand, the maturation spindles of the Chaetopterus egg are relatively small. Thus, Lillie (1906) shows the fully formed maturation spindle of the Chaetopterus egg as small. In his Figures 3 and 4, the distance between the centers of its two centrospheres is only one-sixth of the diameter of the egg (Compare also Mead, 1898). Lillie (1906) showed that the cortex of the Chaetopterus egg was relatively viscous, containing granules which did not move when the egg was subjected to reasonably strong centrifugal force. We conceive, therefore, of the maturation spindles of the Chaetopterus egg as being relatively small bodies only several times as long as the cortical layer is thick and extending only a relatively short distance into the fluid region of the egg.

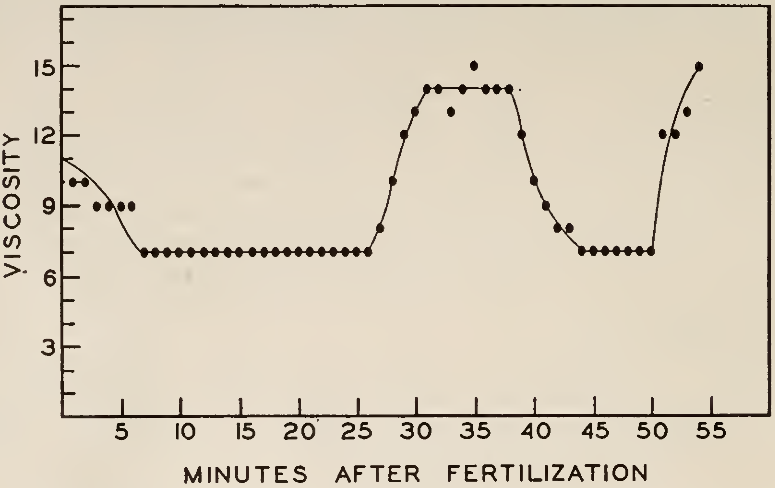

FIGURE 1. Protoplasmic viscosity changes in the egg of Chaetopterus during the time between fertilization and first cleavage.

Following the maturation divisions, the protoplasm undergoes a sharp increase in viscosity. Our curve shows it to be approximately a two-fold increase. Some of our data indicated a somewhat greater change, but we preferred a conservative estimate. The viscosity increase is followed by a decrease in viscosity, and then just before cleavage, the viscosity increases sharply again. These major changes in protoplasmic viscosity are related to the process of mitosis. In earlier work on the eggs of Cumingia and Arbacia, it was found that "the appearance of a spindle is preceded by an increase in viscosity and followed by a decrease in viscosity." It is of interest now to inquire as to whether the same correlations exist for the Chaetopterus egg.

Because of the fact that the Chaetopterus egg is one of the few invertebrate eggs that can conveniently be studied at Woods Hole, and because also of the present great interest in cell division, it was thought worth while to establish a complete time record of the mitotic changes in this egg as they occur during the interval between fertilization and first cleavage. One difficulty in presenting such a time record is the uncertainty of terminology. Mitosis is a continuous process and the various stages of this process can not be sharply delimited from each other. Moreover, not all authorities on mitosis agree in the way they define the stages. And even if a definition is rather uniformly followed, it is not always easy to apply it in such a way as to give a clear-cut decision as to when one stage ends and another begins. Thus, the telophase may be defined as the stage in which the chromosomes reach the poles of the spindle and begin to transform into vesicles or other structures characteristic of the resting stage. Now on a time basis, these two processes may not be simultaneous, and if one seeks to make sharp time distinctions, one must choose either the one or the other. Furthermore, the situation is complicated by the rapid succession of mitoses in an egg cell. Thus there may be no resting stage at all between two successive divisions, and there may not even be a complete telophase between the first and second maturation divisions.

For us it seemed wisest, if we were to present our results in tabular form, to make arbitrary criteria and distinctions. For our purposes, we shall define the metaphase as the stage in which the chromosomes are arranged along the equatorial plate of the spindle. We regard the anaphase as beginning as soon as the chromosomes have divided sufficiently so that we can see a space between the two groups of chromosomes. During anaphase, the two sets of chromosomes migrate toward the poles. It is hard to tell exactly when they have reached the poles. Accordingly, for this material, we chose as the distinction between anaphase and telophase, the moment when at least some of the chromosomes show signs of vesiculation. Actually, this distinction may depend to a slight extent on the depth to which the sections have been stained, but the difference between lightly and darkly stained sections does not appear to be great. On the basis of these distinctions, it was usually not too hard for us to tell when the egg cells were in metaphase, anaphase, or telophase. Prophases were more difficult. At the end of the first maturation division, after the first polar body has been separated off, there is an intermediate series of stages which are hard to classify. The polar body is given off 9 minutes after fertilization. At 10, 11, and 12 minutes after fertilization, one typically sees remnants of the first maturation spindle. At these times, the chromosomes are not vesiculated, so that according to our previous definition, it is not proper to call this stage a telophase. We might refer to it as an interphase, but we prefer to consider it as a late anaphase. At 13 minutes after fertilization, many half spindles appear in the sections. Following this is a stage in which the second maturation spindle appears; typically it lies in a plane perpendicular to the radius of the egg.

The chromosomes are frequently scattered along this second maturation spindle. This stage we refer to as the prophase of the second maturation spindle. The spindle then turns so that at metaphase it is perpendicular to the surface of the egg.

The second polar body is given off at 23 minutes after fertilization. The egg chromosomes now go into a very definite telophase stage and form discrete vacuoles. The egg nucleus is irregular in shape and may look like a bunch of grapes. At this stage, the male pronucleus, in sympathy as it were, may also become lobulated. There is thus a very definite telophase stage (at least in so far as the egg pronucleus is concerned). But between this telophase stage and the late prophase stage of the cleavage mitosis, it is hard to find distinctions which can be used for the purposes of our time scale. The male and the female pronuclei approach each other. There is thus a stage in which the pronuclei are separate and a stage when they are apposed. Usually, by the time they are apposed, the vesicular lobate appearance of the female pronucleus has disappeared so that this nucleus is now a smoothly spherical body with its chromatin either in a resting stage or in a condition indicative of an early prophase. There are exceptional cases in which the female pronucleus preserves its telophase appearance even though it is close to the male pronucleus. Then comes a stage in which the two pronuclei are fused together, or at least are apparently fused together. Mead (1898) says that an actual fusion of the pronuclei does not occur, but in many instances we were not able to detect a line of demarcation between the two pronuclei; and indeed in Mead's Figure 40, such a demarcation line is questionable. Accordingly, we distinguished a fusion nucleus stage. During this stage, the condition of the nucleus is almost certainly what most authors would call early prophase. There then comes a stage in which the nuclear membrane gradually breaks down and the chromosomes are arranged along the developing mitotic spindle; this stage is called late prophase.

In our time schedule, therefore, we distinguished the following stages: 1st maturation metaphase, 1st maturation anaphase, 2nd maturation prophase, 2nd maturation metaphase, 2nd maturation anaphase, 2nd maturation telophase, pronuclei separate, pronuclei apposed, fusion nucleus, late prophase of cleavage mitosis, metaphase, anaphase, and telophase (of cleavage mitosis). These stages are abbreviated in the headings of the table which gives a record of our findings. The data for the table were collected from a study of the slides prepared as described previously. For each minute of the time between fertilization and cleavage, we counted 20 cases in which the condition seemed clear in terms of one of the above-mentioned categories. The work was shared, and each of us made ten counts for each minute. On comparing our results, we found essential agreement.

The stages listed in our table do not give information on one point of considerable importance. They do not indicate at what moment the cleavage spindle first makes its appearance. After careful study of the sectioned material we have decided that the following series of events occurs. At 30 and 31 minutes after fertilization, the two pronuclei begin to come close to each other. There is at this time a large sperm aster with a large centrosphere. As the two pronuclei come still closer to each other (at 32 and 33 minutes after fertilization), between them they squeeze the centrosphere of the sperm aster into an elongated shape, so that it may form a narrow band between the two. This is the stage illustrated in Figure 39 of Mead (1898). This elongated centrosphere, with its astral rays divided into two groups stretching well out into the cytoplasm, is not the definitive mitotic spindle, as subsequent stages indicate. Nevertheless, the line connecting the two sets of astral rays and the line along which the pronuclei fuse is almost always in the direction of the future spindle, for this line is typically perpendicular

TABLE I

Mitotic stages in the Chaetopterus egg as a function of time (minutes) at 21° C. Further explanation in text

Time	1st M	1st A	2nd P	2nd M	2nd A	2nd T	PNS	PNA	FN	LP	M	A	T
0	20												
1	20												
2	20												
3	20												
4	20												
5	20												
6	19	1											
7	12	8											
8	3	17											
9		20*											
10		20											
11		20											
12		19	1										
13		15	5										
14		8	8	4									
15			9	11									
16			3	17									
17				20									
18				19	1								
19				16	4								
20				13	7								
21				6	14								
22				1	19								
23					20*								
24					18	2							
25					8	12							
26						20							
27						18	2						
28						15	5						
29						14	4	2					

* Indicates time of appearance of 1st and 2nd polar bodies.

TABLE I—*Continued*

Time	1st M	1st A	2nd P	2nd M	2nd A	2nd T	PNS	PNA	FN	LP	M	A	T
30						8	9	3					
31						2	7	11					
32						1	5	14					
33							1	19					
34								20					
35								20					
36								9	11				
37								8	12				
38								3	17				
39								4	16				
40									13	7			
41									5	15			
42									4	15	1		
43										16	4		
44										15	5		
45										3	17		
46											19	1	
47											20		
48											18	2	
49											16	4	
50											3	17	
51												20	
52												20	
53												16	4
54												6	14
55												1	19
56													20

to the egg axis as indicated by the position of the polar bodies. This is also shown in Mead's Figure 39. Although at 32 and 33 minutes after fertilization, the astral rays are well developed, subsequently they seem to fade, so that 35 minutes after fertilization the asters either do not appear at all, or if present, they are faint. At this time there is no spindle. In the next three or four minutes, one occasionally sees instances of a double aster at one side of the fusion nucleus with what is apparently an embryonic spindle being stretched out between the two asters. Whether this is a general condition or not, only further study can decide. On one point we are certain, the definitive mitotic spindle does not appear until approximately 40 minutes after fertilization. In our study of eggs fixed at 40 minutes after fertilization, we found 15 out of 20 with the fusion nucleus elongated and pointed at its ends. In the pointed ends of these nuclei, spindle fibers show in 7 out of the 15 cases. Thus 7 out of 20 cells showed a true spindle. Probably this is somewhat less than the true proportion, for a nucleus might well be cut so that spindle fibers though present would not be visible. As far as our observations go, they indicate rather clearly that the moment at which the definitive mitotic spindle appears is 40 minutes after fertilization.

Let us now attempt to correlate our viscosity curve with the mitotic changes as shown by our observations of the fixed material. The viscosity curve shows a minimum of viscosity from 44 minutes after fertilization to 50 minutes after fertilization. This is almost exactly the time during which the cell is in metaphase, for the table shows the metaphase period to extend from 45 to 49 minutes after fertilization. We have chosen as the moment at which the definitive mitotic spindle appears as 40 minutes after fertilization. This is essentially simultaneous with the moment at which the viscosity of the protoplasm begins to drop.

We conclude therefore that the appearance of the cleavage spindle in the Chaetopterus egg is preceded by a period in which the protoplasm is relatively viscous. As soon as the spindle is formed, the viscosity drops. The metaphase is the stage at which the viscosity of the protoplasm is at a minimum.

Finally, we should like to express our admiration of the cytological study made by Mead in 1898. In general we confirm his findings. There are one or two minor points in which we might differ. In his Figure 46, which represents what we would call an anaphase, he shows some bodies in the equatorial plane of the spindle; these he calls nucleoli. Lillie (1906) in his Figure 25 illlustrates similar bodies which he labels as "chromatin masses cut off from the chromosomes." We have frequently seen these bodies in the equatorial plane of the spindle during the anaphase. However, our sections seem to indicate that they are neither nucleoli or chromosome fragments, but rather cytoplasmic granules which have pushed their way into the equatorial plane of the spindle. If this is correct, it is an observation which may have some importance in the interpretation of the mitotic spindle, but we made no careful study of the phenomenon. We should merely like to call the attention of the cytologists to it.

Discussion

Our results provide a suitable basis for further work on the protoplasm of the dividing Chaetopterus egg, and we hope in the future to study the action of radiation and other agents in terms of their effect on the protoplasmic viscosity.

The viscosity curve that we have plotted for the Chaetopterus egg is essentially the same as that reported earlier for Arbacia and Cumingia. Heilbrunn (1921) stated that "The viscosity changes in Arbacia and Cumingia are absolutely parallel. In each case the appearance of a spindle is preceded by an increase in viscosity and followed by a decrease in viscosity. And in both eggs division of the cell is immediately preceded by a viscosity increase." As a matter of fact, all authors who have made objective measurements of protoplasmic viscosity during mitosis are in substantial agreement. A survey of much of the literature is given by Carlson (1946).

If one excludes the work done by subjective and non-quantitative methods, there is only one discordant paper. Fry and Parks in 1934 published what we believe to be a masterpiece of distortion. They made a few centrifuge measurements and then used Heilbrunn's data in plotting their curves, stating that Heilbrunn's measurements were more complete and accurate than their own. After doing this, they insist in a final discussion that Heilbrunn is wrong. They reach this strange conclusion by misquoting and distorting the views not only of Heilbrunn but also of almost every other worker in the field. Actually, though Fry and Parks claimed to have copied Heilbrunn's curves, this is not exactly true. The rise in viscosity which Heilbrunn found to occur in the Arbacia egg ten minutes after fertilization (for a cleavage time of 50 minutes) is shifted by Fry and Parks so that it occurs approximately seven or eight minutes after fertilization (for a cleavage time of 67 minutes). Thus in Heilbrunn's curve, viscosity rises only after one-fifth of the time between fertilization and cleavage has elapsed, whereas in the curve stated by Fry and Parks to be a copy of Heilbrunn's curve, the rise occurs when about one-ninth of the time between cleavage and fertilization has elapsed. Needless to say, this shift favors the interpretation Fry and Parks endorse. Moreover, the final upsweep of the Arbacia curve is shifted so as to make the metaphase of division come in a period of high rather than low viscosity. Fry and Parks claim to find agreement between their curves, which they state to be Heilbrunn's curves, and Chambers' opinions on viscosity change during mitosis, opinions based on subjective microdissection studies of various species of eggs at uncertain times. This they do in order to make Heilbrunn's curves fit what Fry and Parks regard as Chambers' opinions. In their Chart 5, Fry and Parks credit Chambers with maintaining that the metaphase is a stage during which the protoplasm is fluid. But this is the exact opposite of what Chambers says. Thus Chambers (1919) states: "The time of appearance of the amphiaster until completion of cleavage lasts from 10 to 15 minutes. The increased viscosity of the egg during the amphiaster stage could be more easily demonstrated by the needle in the eggs of Echinarachnius and Cerebratulus than in those of Arbacia."

The facts of the case are as we have stated them, and no amount of distortion can hide the fact that the appearance of the mitotic spindle is preceded by a stage of high viscosity and followed by a stage of low viscosity. Heilbrunn (1921) suggested that "it is as though the spindle were coagulated out of the protoplasm." Recent work has indicated that in some types of proteins, gelation may result in the formation of a spindle-shaped structure called a tactoid (Bernal and Fankuchen, 1941). Bernal (1940) believes that the spindle is actually a tactoid.

Perhaps there are other correlations that may be made between changes in the protoplasm and the series of viscosity changes that we have described. For one thing, the stage of increasing viscosity occurs at a time when water is being taken from the cytoplasm by the enlarging pronuclei. Then, when the spindle appears, the nuclear membrane breaks down and this might involve an increase in the water content of the cytoplasm. Carlson (1946) suggests that changes in the viscosity of protoplasm during mitosis may be due to changes in the nucleic acid content of the cytoplasm. He thinks that a high content of nucleic acid in the cytoplasm would tend to produce a high viscosity. Carlson states that Brachet and also Painter found the cytoplasmic nucleic acids abundant in early prophase, less abundant or entirely absent from late prophase through anaphase, and increasing in amount following division; but in the papers cited by Carlson it is not possible for us to find any data on the changes in nucleic acid content of the cytoplasm during various stages of mitosis. That there is an exact correlation between the amount of cytoplasmic nucleic acid and the protoplasmic viscosity is very doubtful, and certainly it has not in any sense been established. For one thing, the unfertilized sea-urchin egg is apparently rich in cytoplasmic nucleic acids (see, for example, Caspersson and Schultz, 1940), and yet this unfertilized egg has a low protoplasmic viscosity (Heilbrunn, 1920).

SUMMARY

1. The viscosity of Chaetopterus egg protoplasm was determined at one minute intervals during the period between fertilization and cleavage.
2. By studying fixed, sectioned and stained material, the course of the mitotic processes in the Chaetopterus egg was followed minute by minute.
3. During the cleavage mitosis, marked changes in protoplasmic viscosity occur, and these are similar to the changes already described for the eggs of Arbacia and Cumingia.
4. The appearance of the mitotic spindle is preceded by an increase in protoplasmic viscosity and is followed by a decrease in protoplasmic viscosity.
5. During the metaphase, the protoplasmic viscosity is low.
6. Just before the cell divides, the protoplasmic viscosity increases markedly.

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INHIBITION OF FERTILIZATION IN ARBACIA BY BLOOD EXTRACTS

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From the time of publication of F. R. Lillie's paper (1914) on fertilization in Arbacia, some embryologists have maintained that the serum of Arbacia blood[1] provided an effective block to fertilization in this and a few other marine invertebrates. Lillie formulated the hypothesis that fertilization in Arbacia was actuated through the conjoining of certain constituents of egg and sperm by a substance called fertilizin, the presence of which in solution could be detected by the agglutinating action which it exerted upon sperm in aqueous suspension (see Tyler, 1948, for recent review of the subject). Asserting that filtered blood of Arbacia was capable of inhibiting fertilization while it did not prevent fertilizin from agglutinating sperm, Lillie linked this inhibitory action into his conception of the mechanism of fertilization by postulating that the serum-inhibitor prevented the uniting of fertilizin with the necessary constituent of the egg.

Oshima (1921) published the results of a few experiments which had motivated him to suggest that an external ("dermal") secretion was responsible for the inhibition observed by Lillie. That Oshima was not prepared to enter a complete denial of Lillie's conclusions is evidenced by his admitting that filtered blood was capable of exhibiting a weak though unpredictable inhibitory influence upon the fertilizability of the egg. Interestingly enough the degree of inhibitory action considered weak by Oshima fell within the range certainly considered significant by Lillie. Furthermore, it is worthy of note that Lillie was not able to offer a satisfactory explanation of the fact that the potency of undiluted blood samples displayed degrees of inhibitory effectiveness varying from zero to one hundred per cent. Nonetheless, largely through the influence of E. E. Just, little or no attention was paid to Oshima's suggestions by the majority of interested embryologists, except, perhaps, for Harvey (1939).

Apparently critical data confirming Lillie's conclusions were brought forth by Just (1922), who, at the same time, brushed aside Oshima's contraindications without any statement that he had attempted to repeat the latter's experiments. Also, Just stated that the most plausible explanation of Oshima's results would depend upon the presence of excretory or defecatory wastes in his solutions.

The matter rested at this point until the summer of 1946 when, at the suggestion of Dr. Albert Tyler, Richard L. Murtland, Albert H. Banner, and the present author [2] collaborated briefly in repeating a few of Lillie's experiments. Becoming interested in the mechanism of this inhibitory action, I carried on alone during the latter part of the summer of 1946 and returned to Woods Hole in the summer of 1947 to proceed with the same problem.

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[1] It has been convenient to use the word blood as a synonym of the term perivisceral fluid, even though strict interpretation may not warrant the practice. For present purposes the words serum and plasma are considered as literal equivalents when applied to Arbacia, since in this organism the clot is believed to be purely of cellular composition. Since Lillie and Just had previously used serum to denote the material obtained from whole blood by clotting, filtering, or centrifugation, I have followed this choice entirely for the virtue of consistency.

Although at the time I was unaware of Oshima's publication, I undertook to verify Lillie's observations before proceeding to a study of the *modus operandi* of the inhibitor. Following Lillie's techniques as closely as possible, I obtained results which corroborated his. Thus, I was convinced that his conclusion to the effect that Arbacia serum contained a factor capable of inhibiting fertilization was valid. But when, during the summer of 1947, I introduced techniques of collecting blood designed to yield uncontaminated samples, I obtained results which revealed that Lillie's original description of the source of the inhibitor must be modified. During the course of this study, it was found that serum samples removed by syringe so that they were uncontaminated with drainage from the exterior of the test did not possess inhibitory activity. Furthermore, it was found that sea water extracts from the tests of intact Arbacia were not capable of inhibiting fertilization. Thus, it became evident that some step in Lillie's technique, which I had followed previously in obtaining corroboratory results, was responsible for the appearance of the inhibitor in the serum samples. Chosen as the most likely cause was the fact that prior to opening the perivisceral cavity both Lillie and Just rinsed their urchins in tap water, presumably to kill any sperm present on the test which would otherwise fertilize samples of eggs. I had noticed that the application of tap water, even when followed by a sea water rinse within a few seconds, caused a yellow substance to appear in the excess water draining from the test of the as yet intact animal. A detailed study of this phenomenon revealed that this yellow exudate was capable of inhibiting fertilization. Additional experiments revealed that the immediate source of this inhibitor was to be found in certain granules or cells located in the tube feet and a few other organs. And, contrary to the findings of Lillie and Just, the ultimate source of the inhibitor was found to be some of the blood cells found in the perivisceral fluid. The present paper gives the details of these experiments.

METHOD

The sea-urchin *Arbacia punctulata* was the principal animal used in these experiments. Perivisceral fluid was removed from Arbacia by methods designed both to permit contamination from the outside and by methods devised to prevent such contamination. In addition, various techniques were devised which might supply information relative to the ultimate source of the inhibitor. Also, one significant step not used by previous workers was added to the routine handling of all samples. Having noted that the pH of sea-urchin blood was lower than that of sea water, and since this in itself may interfere with fertilization (Tyler and Scheer, 1937), it was decided that the pH of all samples should be adjusted to that of sea water. Moreover, in order to obviate any modification of results arising from undue concentrations of egg or sperm secretions, all samples containing gametes were discarded.

In most experiments one drop each of eggs and sperms were introduced into 2 cc. of fluid, be it sea water or extract, in Syracuse dishes. In each instance the eggs from a single female were used for each series of experiments. The average concentration of eggs in suspension was found by actual count to be between 2200 and 2500 eggs per drop. Fresh sperm suspensions were used for fertilizations and were made by diluting one drop of dry sperm with the equivalent of 99 drops of sea water. The various fluids were tested in serial two-fold dilutions of 2 cc. down to 64-fold. Determinations of the percentage of fertilization were made by actual counts under low magnification from three to five hours after insemination. The practice of first scanning the dish and then counting between four and five hundred eggs along two diameters was followed consistently.

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[2] Three members of the 1946 Embryology Class of the Marine Biological Laboratory.

Most of the experiments with differently prepared fluids were run simultaneously, as is indicated by similar dates in the tables.

Experiments

Series I. This experiment was carried out essentially as outlined by Lillie and Just, as follows: (1) Arbacia rinsed in tap water for a few seconds, shaken and rinsed in filtered sea water; (2) animals permitted to drain, cut made with scissors around peristome, and fluid drained into Syracuse dish; (3) clot permitted to form, checked for presence of gametes, then filtered; and finally (4) samples were centrifuged lightly and the pH adjusted to that of sea water. The fluid was then used undiluted, or diluted as described above. In each instance the fluid obtained in this way had a yellowish tinge.

This series of experiments, involving a total of 42 animals, was repeated seven times between July 8 and August 2, 1947. From the results tabulated in the left half of Table I it can be observed that this solution was effective in blocking fertilization when used undiluted. An average of approximately 3 per cent fertilizations was obtained in undiluted fluids as compared to nearly 100 per cent fertilizations of eggs in sea water controls. A summary of part of Lillie's work (1914), in which undiluted fluid collected from 50 Arbacia in the same manner and within the above dates was used, gives an average of 50 per cent fertilizations, as compared to 97 per cent in the sea water controls. The apartness of our results can be explained in part by the fact that his samples were used individually, while in my experiments fluid from all individuals was pooled before being tested. For example, Lillie's data show that the serum obtained by him from one individual contained no inhibitor, while the serum from another contained enough to inhibit all eggs tested. This would yield an average of 50 per cent inhibition. On the other hand, if these two samples had been pooled before being tested, it is possible that sufficient inhibitor would be present in the mixture to give complete inhibition. A comparison of the effects of diluting the serum show this conjecture to be valid. Thus, the percentage of fertilization increased when the fluid collected on August 2 was diluted, as follows:

Percentage serum (in sea water)	100	50	25	12.5	6.2
Percentage fertilization	0	16	68	99	100

In this experiment I obtained an average of 32 per cent inhibition with a 25 per cent solution of serum, and Lillie's data show that he obtained 30 per cent inhibition when using a 20 per cent solution of serum obtained in the same manner.

Series II. The tap water rinse was eliminated in this experiment; otherwise all procedures were the same as those outlined in Series I. Again, fluid from a total of 42 animals was tested on seven occasions between July 10 and August 3, 1947. The fluid in each case was clear, not yellow.

The results, as tabulated in the right half of Table I, offer a marked contrast to those of Series I. There is no significant difference between the percentages of fertilizations obtained from eggs inseminated in undiluted serum and those inseminated in sea water (both yielding approximately 99 per cent fertilizations). Hence it became apparent that the application of tap water was linked in some manner with the appearance of the inhibitor. Additional experiments were performed in order to determine whether it was being liberated into the serum from within the animal or from the outside.

TABLE I

Serum obtained by cutting peristome

With tap water rinse (Series I)					Without tap water rinse (Series II)				
July	pH	Adjusted pH	Percentage fertilizations		July	pH	Adjusted pH	Percentage fertilizations	
			Serum (100%)	Sea water				Serum (100%)	Sea water
8	7.5	8.0	0	100	10	7.6	8.0	100	100
10	7.4	8.0	0.5	100	12	7.6	7.9	99	99
12	7.6	8.0	0	99	14	7.7	8.0	100	100
14	7.6	8.0	0	100	21	7.5	7.9	100	100
26	7.2	8.0	10	100	26	7.5	8.0	95	100
27	7.5	8.0	5	99	Aug. 2	7.6	8.0	100	100
Aug. 2	7.5	8.0	0	100	3	7.0	8.0	100	100

Series III. In order to circumvent contamination of serum with external drainage, the fluid was withdrawn by means of a 10 cc. syringe equipped with a No. 22 gauge hypodermic needle. The needle was introduced into the perivisceral cavity through the peristome about 3 mm. from Aristotle's lantern. Care had to be exercised to keep the needle from penetrating the gonads and to prevent the plunger from crushing blood cells when fluid was expelled from the syringe. When the proper depth of penetration had been determined, the needle was ensheathed with rubber tubing long enough to stop it at the desired level. All animals used in this experiment were rinsed momentarily in tap water and then in sea water prior to withdrawing the fluid. Subsequently, the fluid was prepared and used exactly as described previously. The fluid in all cases was clear, not yellow.

Eight experiments were performed with serum collected in this manner between July 8 and August 3. The data from this series are tabulated in Table II. An average of 99.1 per cent fertilizations was obtained from eggs fertilized in all undiluted samples of this serum. This evidence, when coupled with the results of Series I, indicated that the inhibitor evoked by tap water drained into the perivisceral fluid from the outside when the latter was collected by cutting the peristome. Moreover, there seems little reason for doubting that these facts explain the large range of variation in potency of inhibitor recorded both by Lillie and Just, because the amount of drainage in their samples would have varied inversely with the time elapsing between rinsing the animals and withdrawing the fluid, and directly with the time required to drain each animal.

Thus far the following facts have been ascertained: (1) that the inhibitor of fertilization is not found in the serum of the intact Arbacia; (2) that tap water causes the inhibitor to appear in samples of serum collected by the method of Lillie and Just (Series I); and (3) that the inhibitor so evoked comes from the outside of the animal.

It is important to note that when inhibition has been observed up to this point perivisceral fluid plus external drainage have been in solution together. Further experiments were performed to reveal whether this complex was necessary for inhibition, or whether the yellow drainage alone was sufficient to cause inhibition of fertilization. The fourth series of experiments was devoted to this problem.

TABLE II

Serum withdrawn by syringe after tap water rinse

(SERIES III)

July	pH	Adjusted pH	Percentage of fertilizations	
			Serum (100%)	Sea water
8	7.5	7.9	100	100
10	7.6	8.0	100	100
12	7.6	7.9	98	99
14	7.7	8.0	99.5	100
17	7.9	7.9	100	100
26	7.5	7.9	95	100
Aug.				
2	7.5	8.0	100	100
3	7.0	8.0	100	100

Series IV. Several Arbacia were washed in filtered sea water to remove debris and wastes, then rinsed under the tap for fifteen seconds, and finally submerged briefly in filtered sea water to correct hypotonicity. After being shaken the animals were placed edgewise in glass funnels fitted with moistened filter paper, and the yellow drainage collected in centrifuge tubes. An equal number of control animals was subjected to the same treatment except that no tap water rinse was administered. Material from any animals that proceeded to defecate or shed gametes was discarded. The animals were left in the funnels a maximum of 20 minutes, or until draining ceased. The filtrate was centrifuged, although no visible separation occurred, and the pH adjusted to that of sea water. The clear, yellow fluid was then used as before.

This experiment was repeated seven times between July 10 and August 2, using a total of 68 animals. The results shown in Table III indicate that this material alone is sufficient to bring about inhibition of fertilization. In only two instances was fertilization obtained in this material when undiluted, and then only an average of 2 per cent of the eggs were activated. These data show also that

TABLE III

(SERIES IV)

Yellow fluid obtained from the test by draining after tap water rinse

July	pH	Adjusted pH	Percentage of fertilizations							
			Fluid dilutions (per cent)						Rinse control 100	Sea water control 100
			100	50	25	12	6	3		
10	7.6	8.1	1	—	—	—	—	—	97	99
12	7.8	8.0	0	—	—	—	—	—	100	100
14	7.9	8.0	0	—	—	—	—	—	100	100
17	7.8	8.1	0	0	0	0	17	100	96	97
19	7.6	7.9	0	0	0	0	0	—	95	98
*27	7.5	7.0	0	0	0	0	0	0		
27	7.5	8.0	3	2	3	3	3	99	100	100
27	7.5	9.0	21	29	10	10	21	84		
Aug. 2	7.6	8.0	0	0	0	0	1	5	100	100

* The experiment of July 27 was carried out at three pH's. The inhibitor's effectiveness is apparently reduced at pH 9. Note also an increase of effectiveness with moderate dilution; this occurred at other pH's as well.

these solutions effectively blocked fertilization in dilutions ranging from 50 to 3 per cent by volume. As a check some of this material was mixed in varying amounts with perivisceral fluid which alone had no effect on fertilization. As was anticipated, the previously impotent serum now became inhibitory to fertilization in proportion to the amount of yellow drainage added (Table IV). No appreciable difference in the inhibitor's activity could be detected between that diluted with sea water and that diluted in serum. Since the liquid draining from the control animals (those not rinsed in tap water) was found to be ineffective in blocking fertilization, it appears unlikely that this inhibition is due to the presence of soluble wastes, at least as proposed by Just in answer to Oshima's report.

TABLE IV

(SERIES IV)

Inhibitor mixed with impotent serum

Percentage of components in mixture		Percentage fertilizations	
Serum	Inhibitor	Mixture	Sea water
95	5	98	100
90	10	78	—
85	15	24	—
80	20	2	—
75	25	0	—
70	30	0	—
50	50	0	100

Series V. Much more potent solutions of inhibitor were obtained by (1) placing urchins directly into distilled water to depths not exceeding the greatest circumference of the shell and permitting them to remain 15 minutes, and (2) adjusting the osmotic value of the solution with sea water concentrated by evaporation. Control animals were placed in sea water to soak for the same period of time as the test animals. The osmotic values of these test solutions were checked by comparing the diameters of test eggs with those of the controls. No significant variations were observed. In addition one control was composed of equal volumes of distilled water and sea water concentrated to half its original volume by evaporation.

Although this experiment was run on several occasions, only one will be described in detail since all were essentially the same. On July 31 half a dozen Arbacia were placed in succession into 40 cc. of distilled water and permitted to remain approximately 5 minutes each. Six control animals were placed into the same volume of sea water in the same manner. The distilled water was immediately colored yellow, while the sea water remained clear and colorless throughout. After filtration, centrifugation, and adjustment of osmotic value, 62 cc. of yellow fluid were obtained. This obviously represents a much greater dilution per animal than in previous experiments. But despite this fact this solution prevented fertilization completely in serial dilutions down to 1 per cent. The sea water in which control animals had stood gave 100 per cent fertilizations, as did the other control solution.

Series VI. In order to narrow down the locus of origin of the inhibitor, five animals were cut into halves along the oral-aboral axis. All internal organs were removed and the inside of the tests scrubbed in sea water with a brush. Following this the sectioned tests were soaked for one hour in sea water, which was not discolored in the process. Then the tests were rinsed for a few seconds in tap water and sea water, and permitted to drain into a clean finger bowl. The drainage was yellow. The spines on the tests were still moving when the fluid was removed after one hour. The pH was adjusted from 7.6 to 8.0, and the material tested. The following results were obtained:

Percentage of extract	100	50	25	12	6	3
Percentage of fertilizations	3	6	3	3	92	100

When the same tests were again rinsed in tap water and sea water and permitted to drain, only 2 cc. of fluid were obtained. When used undiluted this second drainage gave 10 per cent aberrant cleavages. Controls gave 98 and 99 per cent fertilizations, respectively. These results provided additional evidence that some external structure was the source of the inhibitor.

Series VII. A study of individual tube feet under the microscope revealed a layer of closely packed, yellow granules or cells just beneath the outer epithelium. These granules maintained their integrity while immersed in sea water. But when the sea water was replaced with tap water, all traces of yellow material disappeared from within the feet. Simultaneously with this disappearance, the water around the feet was colored bright yellow. It is important perhaps to note that this material diffused through the outer epithelium and did not pass into the lumen of the foot. Tests run on this yellow material proved that it possessed the property of inhibiting fertilization in a manner similar to that observed previously. One experiment will be described in greater detail.

Part of the tube feet from the oral hemispheres of four Arbacia were removed. This was done by letting the animal attach to a glass plate and then pulling the plate away. The tube feet were soaked in several rinses of sea water, covered momentarily with tap water and then placed into 4 cc. of clean sea water. The latter water was immediately colored yellow. After five minutes the supernatant fluid was decanted and its volume noted to be 5 cc. It was then filtered and the pH raised from 7.6 to 8.0. It is worthy of note that this extract was brighter yellow than that obtained by placing the whole animal in distilled water. The color of the extract deepened to a certain extent when the pH was elevated. Interestingly enough I found subsequently that it became colorless at pH 4 and below, and a darker yellow at pH 8 and above.

This tube-foot extract proved to be very effective at blocking fertilization. In concentrations running from 100 to 12 per cent no fertilizations occurred, and only 1 per cent of the eggs was fertilized in dishes containing as little as 3 per cent extract in sea water. This degree of effectiveness is made more remarkable by the fact that because of the dilution intrinsic to the method of extraction the extract represented only 20 per cent by volume of the inhibitor solution that exuded from the tube feet. Hence, the 12 per cent solution in the series of 2 cc. dilutions would actually contain a maximum of 0.025 cc. of inhibitor, or approximately 1.25×10^{-5} cc. per egg.

Because the color of this extract was not the same shade as that obtained when intact animals were used, I searched for other sources. It was found that spines gave forth a small amount of inhibitor, but only from their bases where epithelium was to be found. The bodies and tips of the spines, which in many instances had no fleshy covering, gave up a purplish substance which had no significant effect on fertilization. When this substance was mixed in small amounts with the extract from tube feet, however, the latter assumed the color of the extract from the intact animals.

Series VIII. Further work revealed that the inhibitor was carried by at least one type of amoebocyte found in the perivisceral fluid. Blood was removed by cutting the peristome, but attention is called to the fact that the animals were not rinsed in tap water. And, instead of filtering the blood as before, the plasma or serum was separated from the cells by light centrifugation and then decanted into clean flasks. At this time, an equal volume of sea water was added to the clot in the tube and the two mixed by shaking and rapid centrifugation. Whereas the plasma was colorless, the supernatant sea water solution was the same bright yellow as the extract from the tube feet. The pH of the two solutions offered an additional point of contrast. Whereas the pH of the plasma was 7, that of the yellow extract was 6.3, despite the fact that the sea water was pH 7.9 at the time of its addition to the clot. It is suggested that this depression of pH was caused by the liberation of the acid contents of the colorless amoebocytes. Before being tested, both solutions were brought up to pH 8.

A further contrast of properties of these two solutions was observed when they were tested: the percentage of fertilization in solutions of plasma equalled that of the controls, in this case 99 per cent; the yellow extract, however, permitted no fertilizations when used undiluted. A two-fold dilution of the extract permitted only 10 per cent of the eggs to be fertilized. These results showed clearly that the inhibitor was carried by certain blood cells, and in such a manner that it did not normally pass from them into the plasma. Attempts to isolate the specific type or types of blood cells that carried the inhibitor were nullified by the fact that no practical method was devised for preventing the blood from clotting. The methods usually employed to prevent clotting of vertebrate blood were found to be of no value. Nonetheless, it was possible to observe microscopically that upon cytolysis the amoebocytes with yellow spherules (for classification of blood cells, see Kindred, 1926) gave up a yellow substance which upon addition of acid became decolorized as does a solution of the inhibitor. Two additional observations also serve to link the inhibitor obtained by methods described previously with that obtained directly from the blood cells.

It was possible to demonstrate that the potency of inhibitor extracts obtained from blood clots of animals that had been soaked previously in distilled water was less than that obtained from untreated animals. For example, blood was removed by syringe from six animals which had been used just previously for obtaining inhibitor by soaking in distilled water (after the method of Series V). The blood was then centrifuged, the plasma decanted and replaced by sea water, and the mixture shaken and centrifuged rapidly. These solutions were tested with the result that no fertilizations were permitted in the extract obtained by soaking in distilled water; 50 per cent fertilizations were obtained from the undiluted sea water extract of the clot; and 100 per cent fertilizations were obtained in the plasma and sea water controls.

The reciprocal of the above was also found to be true, viz., that animals from which all possible perivisceral fluid had been removed by syringe produced weaker solutions of inhibitor obtained by application of tap water (after the method of Series IV). I took six animals from which perivsceral fluid had just been removed by syringe, and rinsed them briefly in tap water and sea water, and then placed them into funnels from which the drainage was collected. This drainage permitted an average of 32 per cent fertilizations when used undiluted.

All of the observations made in this series of experiments lend some support to the opinion that the yellow granules observed in the tube feet may actually be yellow amoebocytes that are free to move between tube feet and the perivisceral cavity.

Series IX. The following experiment was devised to show whether or not the inhibitory effect of blood extracts upon eggs was reversible. One drop of eggs was placed into 2 cc. of undiluted inhibitor solution contained in each of six Syracuse dishes. After insemination the dishes were placed in running sea water on the water table. No fertilizations resulted in any of the dishes. At the end of two hours, the inhibitor solution was pipetted from one of the dishes and the eggs washed twice in fresh sea water and then reinseminated. Eggs in the remaining dishes were handled in the same manner 4, 8, 12, 14 and 24 hours after being introduced into the inhibitor solution. A series of six control dishes contained approximately the same number of eggs in 2 cc. of sea water; these were fertilized in series after the same intervals of time. The results are tabulated in Table V. Although there are some indications that some eggs were damaged by standing in the inhibitor solution, there is definite evidence that this blocking of fertilization is reversible.

TABLE V

(SERIES IX)

Fertilization of blocked eggs after washing

Exposure to inhibitor (hours)	Percentage fertilizations		
	Before washing	After washing	Controls
2	0	97	100
4	0	92	98
8	0	95	96
12	0	*90	94
14	0	†99	98
24	0	90	93

* About 30 per cent exhibited polyspermy.
† About 10 per cent aberrant cleavages.

Series X. Eggs that were observed to develop in various dilutions of inhibitor had indicated that post-fertilization developmental processes were not appreciably affected, but one experiment was run to test this point more effectively. Eggs were inseminated in sea water and transferred as quickly as possible into dishes containing undiluted inhibitor. Subsequent examinations revealed no significant differences between those samples of eggs that were placed in inhibitor and those that remained in the sea water. The zygotes were kept in the original inhibitor solution until they had reached the swimming stage. At this time they were transferred to sea water and carried on to the pluteus stage. Two observations were made during this time: (1) there were some indications that the rate of development was retarded slightly by the inhibitor, and (2) that the plutei developing from inhibitor-treated zygotes were smaller than the controls. These observations were not investigated further.

DISCUSSION

The results obtained from Series II and III of experiments refute the validity of the conclusion of Lillie and Just that the serum of Arbacia blood normally contains an inhibitor to fertilization. Although Series VIII showed that the inhibitor was carried by certain blood cells, the results of Series II indicated that the inhibitor did not leave the blood cells to enter the serum. In fact, the inhibitor was obtained from the cells in Series VIII only after vigorous shaking and rapid centrifugation. Since it has been shown that the inhibitor can be evoked by the application of tap water to the outside of the animal, it may be concluded that the inhibitor observed by them entered their samples from the outside of their urchins as a result of the application of tap water. Furthermore, the supposed variations in potency of the inhibitor reported by Lillie and Just were shown to be illusory by the results of Series I and III.

Certainly the property of inhibition is not of itself particularly interesting for no doubt a large number of substances could be used to inhibit fertilization in Arbacia, but most of them would very likely be inimical in one way or another to the gametes. Therefore, the fact that eggs appear to be fundamentally unharmed by exposures to this natural inhibitor ranging from a few seconds to many hours serves to heighten one's interest. It has been shown that eggs which have remained blocked up to 24 hours in this inhibitor can be fertilized, provided they are washed thoroughly in sea water and reinseminated. Moreover, Just (1922) reported that he obtained development in blocked eggs (inseminated in inhibitor solution) without reinsemination, so long as the eggs were washed within two hours after fertilization.

Except for a slight depression of the rate of development, this inhibitor exerted no appreciable influence upon post-fertilization changes in the egg. Eggs that were fertilized at one instant and transferred immediately to inhibitor proceeded to develop into normal blastulae; yet when eggs were introduced into potent inhibitor and sperm added as quickly as possible blocking was complete. This latter observation supports the hypothesis that the inhibitor acts at the surface of the egg. Other evidence may be brought to bear on this point.

The inhibitor appeared to remove part of the egg's jelly layer, in proportion to concentration or to the duration of exposure. Inferred at first from the observation that eggs tended during the period of contact with inhibitor to aggregate more compactly than eggs in sea water, this conclusion was strengthened by the addition of dilute solutions of Janus Green B. Furthermore, the jelly layer of Chaetopterus eggs exhibited a marked affinity for the Arbacia inhibitor by staining a deep yellow during exposure, but the jelly layer was not removed by it. It is interesting to note also that this yellow cast was not removed by subsequent washings. Because the inhibitor obtained from Arbacia was observed to prevent fertilization of Chaetopterus eggs, it is unfortunate that no attempt was made to determine their fertilizability after washing. This might well have revealed whether the inhibitor itself is yellow or is only associated with the pigment in solution. While referring to associated species, it is appropriate to record that the Arbacia inhibitor does suppress fertilization in the sand-dollar, Echinarachnius parma. This fact was reported by all previous workers. In addition, Just (1923) stated that the blood of this sand-dollar blocked the fertilization of its eggs. It is possible, however, that this observation is subject to the same criticism herein advanced against his interpretation of the Arbacia inhibitor, because I observed that tap water evoked a similar response from Echinarachnius. Unfortunately, I could find no complete description of the method he used in obtaining this fluid.

Normal fertilization membranes were seldom observed on eggs that were fertilized in fresh sea water after prolonged exposures to the inhibitor. It is possible that this condition resulted from simple aging of the eggs. But in some instances no membranes could be observed even after the eggs began to cleave. That this was not tight membrane development is attested to by those extreme cases in which the blastomeres rounded up and were as easily separable as those of eggs treated with Ca-free sea water.

It was more difficult to observe definitive effects of the inhibitor on sperm. Little positive evidence as yet obtained rules out the possibility that the inhibitor acts directly upon the sperm. But this position could be rendered less tenable by several observations. In the first place, sperms appeared to be stimulated to greater activity when in the presence of inhibitor; and, secondly, they continued to move about inhibited eggs long after all evidence of motility of sperm had disappeared in the controls. One point in this connection that might be of value in future work is the fact that the sperms which persisted in activity longest appeared to have lost their ability to attach to the egg. Thus, they moved about aimlessly among the eggs without attempting to penetrate. Finally, Just (1922) reported the actual penetration of sperms into the cortex and cytoplasm of blocked eggs. Presumably these were the sperms that were able to consummate fertilization when such blocked eggs were washed within the two-hour limit but not reinseminated.

Differences of opinion have arisen concerning the interaction, if any, between inhibitor and fertilizin. Lillie (1914) concluded that the effects of the inhibitor could be nullified by mixing it with fertilizin. One cannot question the data from which he drew this conclusion, but it appears to the present author that the method that he used to obtain neutralization of the inhibitor permits another interpretation of his data. In order to combine fertilizin with inhibitor. Lillie mixed serum and whole eggs in the ratio of two parts serum to one part eggs. Time intervening, the mixture was filtered and the filtrate tested for inhibitory activity. His data show that the untreated serum permitted only 0.5 per cent of the eggs tested to be fertilized, while the treated serum permitted 99.0 per cent of the eggs tested to be fertilized. Lillie concluded that the fertilizin had neutralized the inhibitor in the serum. But it is possible that little or no inhibitor was left in the filtrate. The basis for this interpretation is supplied by an experiment not previously described.

I mixed 4 cc. of inhibitor solution, obtained in the manner of Lillie, with 2 cc. of a suspension of eggs computed to contain approximately 0.75 cc. of sea water (Solution 1). Another 4 cc. of the same inhibitor were mixed with 0.75 cc. of sea water (Solution 2). A third solution was prepared by adding 2 cc. of strong egg-water (known to agglutinate sperm) with another 2 cc. of the inhibitor solution (Solution 3). After an interval of twenty minutes, all tubes were centrifuged lightly and 2 cc. samples were removed carefully from the top of each tube, and tested in the usual manner along with sea water controls. The following results were obtained: Solution 1 gave 75 per cent fertilization; Solutions 2 and 3 gave 0.0 per cent fertilizations; and 99 per cent of the eggs in the sea water controls were fertilized. These data suggested that the inhibitor combined in some manner with the eggs. Also, the fact that no fertilization occurred in the mixture of inhibitor and egg-water (Solution 3) supports the contention that the fertilizin does not neutralize the inhibitor, at least in the same sense of the word as used by Lillie.

The results of this experiment (particularly from Solution 1) provide a tentative explanation of the retarded activity of eggs that have stood in dilutions of inhibitor for some time after insemination. It is obvious that individual eggs in any sample are affected differentially by the inhibitor; otherwise there could be no explanation of the interesting fact that in dilutions of inhibitor some eggs are fertilized while others are not. Furthermore, one should recall that blocked eggs may be reversed to a state of fertilizability by soaking them in sea water. Therefore, it is possible that eggs which at first have the minimum of inhibitor necessary to prevent fertilization give this up slowly when the diffusion gradient has reversed, as the result of the greater affinity of other eggs for inhibitor. Just what conditions in or on the egg account for this differential reaction to inhibitor, I cannot say.

Although it appeared that fertilizin exerted no appreciable influence upon the activity of the inhibitor, some evidence was obtained which indicated that a reverse influence did exist. On the one hand, as indicated in the introductory paragraph of this report, Lillie asserted that the inhibitor did not reduce the ability of fertilizin to agglutinate sperms. On the other hand, I found that concentrated solutions of inhibitor, when mixed in the ratio of 1 : 3 with egg-water capable of agglutinating sperm, would reduce the time required for reversal of agglutination. When these substances were mixed in equal parts, the egg-water lost its ability to bring about agglutination.

An interesting aspect of this problem is revealed by the close parallel between certain properties shared by the inhibitor and material extracted by Tyler (1940) from Arbacia eggs. Both of these materials are yellow; both appear to negate the sperm-agglutinating power of filtered egg suspension; both exhibit tendencies to cause clumping of eggs; and, under certain conditions, both reduce the fertilizability of eggs. The material extracted from eggs, however, produces a visible precipitation membrane on the egg's jelly layer; no membrane of this type has as yet been observed upon applying the blood inhibitor. Nonetheless, this parallelism between the properties of these two extracts is such that further study is indicated.

SUMMARY

1. Whole perivisceral fluid (blood) of Arbacia contains a substance capable of inhibiting fertilization.

2. Contrary to the conclusions of previous investigators, this inhibitor is not normally present in the serum. Rather certain blood cells, particularly the amoebocytes with yellow spherules, are the ultimate source of the inhibitor.

3. The inhibitor believed by Lillie and Just to be found in the serum of Arbacia blood actually entered their samples as a contaminant from the outside of their animals.

4. The external application of tap water causes the inhibitor to appear in the drainage from the test. Under these conditions the inhibitor emanates from yellow bodies found in the hypodermis of the tube feet.

5. The supposedly variable potency of inhibitor reported by previous workers can be explained by the technique used in obtaining samples, and the methods used in testing its strength. In reality former workers were testing inhibitor in varying dilutions rather than testing the potency of a standard amount of inhibitor.

6. This inhibitor does react with the egg's jelly layer and can modify the fertilization membrane in proportion to concentration and duration of exposure. Eggs that are inhibited for short intervals of time (1–4 hours) can be fertilized and will develop normally (i.e. with membranes, etc.), provided they are rinsed thoroughly in fresh sea water.

7. Fertilizin is believed to have little influence on the activity of the inhibitor beyond a simple dilution effect. On the other hand, the sperm-agglutinating power of fertilizin-bearing solutions can be reduced or nullified by the addition of sufficient inhibitor.

8. It is suggested that this blood inhibitor may be related to an egg-agglutinin extracted from the egg itself.

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CHANGES IN DENSITY, WEIGHT, CHLORIDE, AND SWIMBLADDER GAS IN THE KILLIFISH, FUNDULUS HETEROCLITUS, IN FRESH WATER AND SEA WATER

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INTRODUCTION

The field of osmotic regulation in aquatic animals has received much attention during the last fifty years. In fish this work has been largely directed toward a study of the euryhaline species such as the eel and salmon (Krogh, 1939). The theories currently accepted for the maintenance of water and salt balance by normal fish in sea water and fresh water were first thoroughly reviewed by Smith (1932), and have been well summarized by Krogh (1939), Baldwin (1940), and Scheer (1948).

The killifish, *Fundulus,* has been used by many investigators, probably because it is one of the few small euryhaline genera which are available in quantity and adapt readily to aquarium life. The most extensive work dealing with the effect of density changes on *Fundulus* and other fish was carried out by Sumner (1905). His experiments are based mainly on viability of groups of fish in various salinities and fresh water. He also measured weight changes and changes in chloride in the water and in the tissues of *Fundulus* resulting from removal from sea water to fresh water and *vice versa.*

The present investigation was designed to obtain serial quantitative measurements of rapid adjustments of a marine fish to fresh water, and so construct a more complete picture of a reaction whose qualitative aspects are already known.

The author is deeply indebted to Dr. F. R. Hayes for invaluable assistance in formulating the problem and also for information and helpful suggestions regarding the measurements of density of fishes.

MATERIAL AND GENERAL PROCEDURE

Live specimens of *Fundulus heteroclitus,* commonly known as killifish, mummichog, or salt water "minnow," were obtained from salt water flats northeast of Halifax, Nova Scotia. This species is normally found in the sea, in estuaries, and in brackish waters. In the laboratory the fish were kept in large glass aquaria (10 inches by 17.5 inches) having a depth of water of 6.5 inches. Stock sea water was obtained from the Northwest Arm, Halifax. The tap water was derived from the Halifax civic water supply. An analysis of the water made in 1940 (Leverin, 1942) from samples at the pumping station is given in Table I.

Experiments were not begun until the fish had been in the laboratory for at least two days. Fish which had been in the laboratory more than a week were not used to begin a series of experiments. The fish were not fed during the period of the experiment, but stock fish were fed every two days on Aylmer's canned beef

TABLE I

Analysis of Halifax water supply, July 1940 (Leverin, 1942)

	Parts per million		Parts per million
Color	30.0	Bicarbonate (HCO_3)	None
Alkalinity as $CaCO_3$	None	Sulphate (SO_4)	6.6
Residue on evaporation dried at 110° C.	30.0	Chloride (Cl)	2.5
Silica (SiO_2)	4.0	Nitrate (NO_3)	0.35
Iron (Fe)	0.05	Total hardness as $CaCO_3$	18.8
Calcium (Ca)	5.7	Calcium hardness	14.3
Magnesium (Mg)	1.1	Magnesium hardness	4.5

TABLE II

Density, weight, chloride and swimbladder gases of F. heteroclitus *in sea water*

Date 1947	Sex	Weight grams	Density (see text)	Chloride m.eq./kilo wet tissue	Swimbladder		
					CO_2 %	O_2 %	Vol. swimbladder / Wt. of fish
Series I							
June 2	♂	8.28		42	6.2	11.9	0.060
	♀	6.34			6.0	10.1	0.040
	♀	4.50		58	2.3	13.1	0.060
4	♀	6.98		55	3.1	10.5	0.063
	♂	7.87		58	1.3	14.2	0.051
7	♀	6.86		66	0	12.6	0.044
	♀	7.15		60	2.7	10.2	0.050
9	♀	3.40	1.017	50	1.2	15.5	0.048
10	♀	6.90	1.027	55	0.6	12.1	0.061
Average		*6.5*		*56*	*2.6*	*12.2*	*0.053*
Series II							
July 21	♀	2.09		50	1.2	15.1	0.033
	♀	1.51		50			
	♂	4.43			3.6	17.3	0.056
	♀	10.18			3.2	14.0	0.054
	♂	1.87			2.3	21.4	0.029
22	♀	1.47		50	3.0	20.0	0.029
23	♀	1.67		55	1.1	15.1	0.031
	♀	1.26	1.026	52	0.8	12.5	0.032
	♂, ♀	1.74		46			
24	♂	1.71		51	1.3	17.5	0.039
Aug. 1	♀	1.54		58	0.5	11.8	0.045
	♀	4.72	1.024	60	0.8	10.7	0.044
	♂	1.00		60	0.2	11.9	0.045
Average		*2.7*		*53*	*1.6*	*15.2*	*0.040*

prepared for babies. Both sexes were used. Most of the fish were sexually mature. The range in weight of the fish was one to twelve grams, but an attempt was made to select fish of approximately the same weight for each series.

In all aquaria the water was circulated and aerated by fine streams of air bubbles. The water was maintained at a pH between 7.0 and 8.5. The temperature varied from 15° C. to 19° C., but changes were taken into account in the density determinations.

Weight and density measurements were made on groups of two or three fish, taken as a unit, before the fish were transferred from sea water to the tap water (soft water) aquarium. The transition from sea water to fresh water, or the reverse, was always direct; no gradual acclimatization was undertaken. After a known number of hours or days the weight and density measurements were again made. The groups of fish were identified by the manner in which the tail fin was clipped.

Swimbladder gases and chloride content of the fish in the sea water aquarium were determined every other day during the course of a series of experiments and provided the data for "normal sea water fish" (Table II). Swimbladder gases and chloride content of the experimental fish in tap water were determined immediately after the weight and density measurements had been made.

METHODS

All weight determinations were made on a chemical balance accurate to 0.01 gram. The fish were blotted with paper towels in order to remove excess water before weighing.

Dry weights are given in Table III. The fish were dried in an electric oven for 14 to 20 hours at 95° C. and then for 1 to 2 hours at 105° to 115° C.

TABLE III

Dry weights of Fundulus heteroclitus

Date 1947	Chloride in water m.eq./liter	Sex	Weight grams	Dry weight % of wet weight
Aug. 6	440 (sea water)	♀	2.20	22.0
		♀	2.28	20.8
		♀	1.06	20.8
		♂	1.05	20.9
		♂	2.52	21.0
				Average 21.1
	After 7 hrs. in less than 1 (tap water)	♀	1.48	19.4
		♂	1.40	20.0
				Average 19.7
		% water taken on in 7 hrs. = 6.6		

The method of Lowndes (1938) was employed to determine the density of the fish. The same weighing bottle (51.27 cc.) was used in all determinations. The method is based on the fact that density of fish $= \frac{\text{weight of fish}}{\text{volume of fish}}$. The weight of the fish was obtained both directly and by calculation from weight in water and volume in water. The volume of the fish in sea water was calculated from a comparison of the chloride content of undiluted sea water with the chloride content of the water which had been decanted from the weighing bottle containing the fish and made up to volume with distilled water.

The volume of fish in fresh water was determined colorimetrically at the suggestion of Dr. Hayes. Measurements were made using aquarium water dyed with trypan blue. The volume was determined by colorimetric comparison of water in the volumetric flask (decanted from weighing bottle with fish and made up to volume) and undiluted dyed aquarium water. A Klett colorimeter was used.

Results obtained by the chloride and colorimetric methods were checked by calculating the density from weight in air and weight in water where density of fish $= \frac{\text{weight in air} \times \text{density of water}}{\text{weight in air} - \text{weight in water}}$. These three procedures for determining and calculating densities were carried out on six separate groups of fish in dyed sea water. The standard error for results from the three methods varied from ± 0.0002 to ± 0.0055 in the six analyses made, showing good agreement of the methods.

After weight and density had been determined the swimbladder gas was withdrawn under water using a hypodermic needle and syringe. The gas was immediately expelled into a Krogh micro-gas-analyzer (Krogh, 1908). The volume of gas was estimated in the bulb of the apparatus which was calibrated in tenths of a cubic centimeter. Estimates of small gas volumes were less reliable than those of large volumes. About 0.08 cc. of the gas was analyzed, carbon dioxide being absorbed by one-fourth normal potassium hydroxide, and oxygen by reduced violet chrome alum, as described by Hayes (1939).

Chloride in water samples was determined by Mohr's method. The chloride content of fish tissues was analyzed by means of Van Slyke and Sendroy's micromethod as given by Peters and Van Slyke (1932). All fish were washed under the tap before chloride analyses were made.

RESULTS AND DISCUSSION

The results reported here are derived from work done in June and July 1947. Two preliminary series (August 1946; May 1947) were also made for which the data are not complete but which show clearly the same trends indicated in the two series presented in this paper. A fifth series in which fish were transferred to tap water containing calcium carbonate gave similar results, although the loss of salt and gain in weight (water) was not as marked (cf. Weil and Pantin, 1931; Pantin, 1931; Breder, 1933).

Series I. *Response of Fundulus heteroclitus to soft tap water*

The average density of the sea water was 1.019, and of the fish in sea water, 1.023 (± 0.0014 for 16 determinations). This average figure for density of fish in sea water is derived from density determinations on fish before introduction to tap water of density 0.997. The course of adjustment is presented in Figure 1. Each point in Figures 1A, 1B, and 2 represents an average of two or three determinations, except points at 18 hours, 72 hours, and 7 days where only one analysis was made. In Figure 1C each point indicates a single density determination using one, two, or three fish.

Within 12 hours the density of the fish decreased to 1.001 and remained between 0.995 and 1.006 for the duration of the experiment. The slight rise in density at 24 hours occurred in both series (Figs. 1C, 3) and might reflect the rapid loss of weight (water) at this time (Figs. 1B, 3).

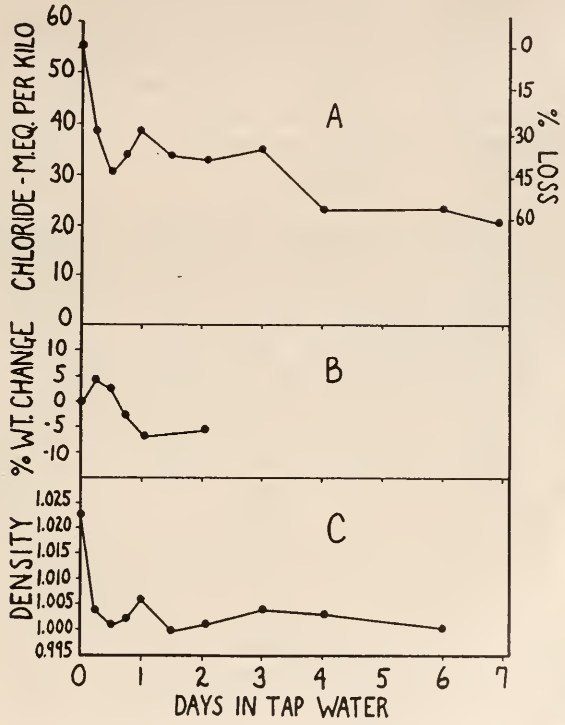

FIGURE 1. Changes in the chloride (A), weight (B), and density (C) in *Fundulus heteroclitus* when transferred directly from sea water to soft tap water.

A rapid gain in weight (4 per cent in 6 hours) follows the introduction of *Fundulus* to fresh water. This phenomenon has been known for some time and Sumner (1905) and Scott (1910) observed an initial gain followed by a loss which persisted throughout the experiment as a result of inanition. The rapid intake of water, probably by way of the gills, is a natural osmotic response to the hypotonic environment. Measurements of the dry weight of two fish at the height of weight gain (7 hours) show a 6.6 per cent increase in water content (Table III). Excretion of water by the kidney may account for the decrease in weight which begins after 8 hours, returning to the initial weight level after about 15 hours and continuing to decrease at a rapid rate for the rest of the first day.

The change in chloride content of the fish is shown in Figure 1A. It should be emphasized that these chloride results apply to the whole fish, and do not reflect the response of any single tissue. By referring to Figures 1A and 1B, it will be seen that an initial dip in the chloride curve corresponds to the curve showing a gain and loss in weight. Since the chloride measurements are calculated on the basis of weight it is likely that the irregularity in the chloride curve during the first day is not real but that chloride is being lost at a constant rate during the rapid gain and loss of water. Sumner (1905) was also aware of this possibility. He found a 25 per cent loss of chloride in *F. heteroclitus* after one day in water having a density of 1.000 or 1.001. The present investigation shows a loss of 30 per cent chloride in one day in water of density 0.997. After the first day chloride continues to be lost gradually during the following 6 days. The fish began to die after the second day, although mortality was not high during the course of the experiments. Of 21 fish in the series, 3 died within the 7 days of experiment. Lack of food may have been a significant factor, although none of the fasting control fish died. Plankton in the unfiltered sea water was available to the controls and no extra demand was being made on their energy as was the case for fish adjusting to fresh water.

From Figure 1 it may be concluded that transfer of *Fundulus* to tap water causes a marked decrease in the density of the fish, an initial increase in weight followed by a decrease, and a gradual loss of tissue chloride. As would be expected in a model osmosis experiment, the water passes through the semipermeable membrane (gills) from the less dense (fresh water) to the more dense (body fluids) solution. Salts (chloride) in the body fluids appear to diffuse out into the water. Sumner (1905) was able to detect this increase in the chloride in fresh water after fish had been introduced from sea water. With regard to water and salts there seems to be little physiological regulation for the first 8 hours by fish introduced directly from sea water to fresh water (Fig. 1). After this period the loss of weight throughout the rest of the first day indicates that the kidneys are probably excreting the excess water taken on by the tissues. Gradual loss of chloride continues throughout the 7 days of the experiment. These changes in salt and water content of the fish contribute to a decrease in the density of the animal. Scott (1910) reported a decrease in the density of the blood of *F. heteroclitus* from 1.0510 to 1.047 after about 8 hours in fresh water. The density of the whole fish at this time, however, has decreased from 1.023 to approximately 1.003 (Fig. 1C).

Adjustment of the density of the fish to fresh water is also assisted by the deposition of gases into the swimbladder, thus making the animal lighter. The function of the swimbladder as an organ for the maintenance of buoyancy in fish is well known, although the exact mechanism whereby fish can separate gases from the blood and deposit them in the swimbladder is still imperfectly understood (Rauther, 1937). The importance of the swimbladder in determining the density of fish is clearly indicated in a paper by Andriaschev (1944) who took density measurements of eight genera of Black Sea fish possessing swimbladders, and eight genera without swimbladders. The range of densities for fish with swimbladders was 1.012–1.021; those without ranged from 1.061 to 1.085.

When *Fundulus* is transferred from sea water directly to tap water, each fish sinks immediately to the bottom for it is heavier than the water. Gas secretion begins and buoyancy is regained after about 24 hours. The equipment for gas secretion and resorption in *Fundulus* consists of a capillary network and "gas gland." The swimbladder of *Fundulus* is physoclistous, i.e., without an open duct leading to the esophagus. Hence all changes in gas content presumably take place by way of the blood.

An inspection of Figure 2C will show that the volume of gas, measured at barometric pressure, increases about 50 per cent in 24 hours. This increase in gas has the effect of inflating the fish and thus decreasing its density. The greatest part of the secreted gas appears to be oxygen which becomes 175 per cent of normal in 24 hours (Fig. 2A). Carbon dioxide increases somewhat in the first 12 hours, decreases again before 24 hours, but shows no marked change thereafter (Figs. 2B, 4).

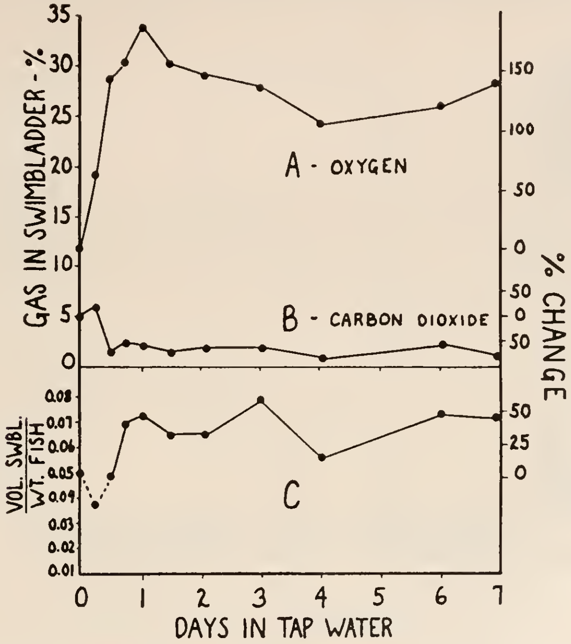

FIGURE 2. Changes in the swimbladder gas of *Fundulus heteroclitus* when transferred directly from sea water to soft tap water.

The data pictured in Figure 2 (and Fig. 4) show that *Fundulus* from sea water adjust to the less dense tap water by increasing the volume of gas in the swimbladder. The greatest part of the secreted gas is oxygen.

Series II. *Response of Fundulus heteroclitus to sea water after two days in soft tap water*

A second series of experiments was run to determine the response to sea water of a group of fish whose fresh water history was known. When the fish are returned to sea water they behave like tops and spin around, head down, at an angle of 45° for about half an hour. The spinning effect is due to the rapid fin movement in an attempt to swim down since they are much lighter than the sea water as a result of the increase in gas volume in fresh water.

The response of the fish on return to sea water is immediate and rapid. The complete history of these fish with respect to change from sea water to fresh water, and the reverse, is presented in Figures 3 and 4. The weight and density measurements on the graphs represent single determinations using two or three individuals. Each point for chloride content and swimbladder gases is the average of results from two fish in the tap water series; in the sea water series, each point represents one fish. The broken line in Figure 3 indicates that the shape of the curve after 24 hours is taken from Figure 1B. In Figure 4 the initial part of the oxygen graph is shown by a broken line because of the large difference between the data whose average is presented by the second point (29 per cent and 44 per cent).

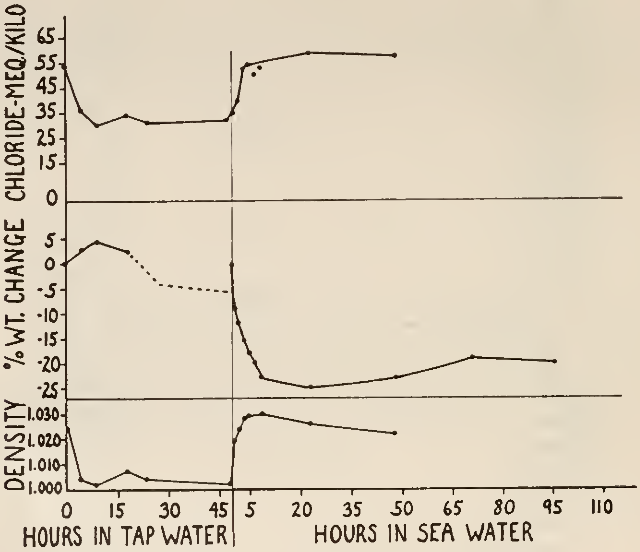

FIGURE 3. Changes in chloride, weight, and density in *Fundulus heteroclitus* transferred from sea water to soft tap water for 2 days, then returned to sea water.

The density, chloride, and swimbladder gases return to normal in 4 hours, although it took 12 to 24 hours for the change from sea to fresh water. Loss of weight, due largely to loss of water, appears to be a continuous process reaching a maximum of − 25 per cent. The rate of passage of water out of the fish is three to four times greater than the rate of imbibition when the fish is put into tap water from sea water. The entire adjustment of the fish to the sea water takes place in six hours, or about four times as quickly as the reverse adjustment from sea water to fresh water.

This difference in rate of change of the factors measured might be explained as follows. When the fish is transferred to fresh water the kidneys may start to function immediately, although the effect of their work is not evident until after

8 hours. If water is being excreted by the kidneys during the period of weight gain the net gain would be the difference between water taken on by the tissues and water excreted by the kidneys. When the fish is returned to sea water, however, the water is passing out of the fish both at the gills and kidneys so that the weight (water) loss could be accomplished more rapidly. Some water, however, probably enters with the chloride.

It is difficult to explain the difference in rate of movement of chloride unless the slower loss of chloride in fresh water is due to the functioning of a salt conservation mechanism in the gills. Krogh has shown that several species of fresh water fish can extract salts from fresh water (Krogh, 1939). A similar mechanism may be functioning here so that the fish can in some way regain a little of the chloride, and so decrease the net chloride loss. When the fish returns to sea water, however, the gradient is such that chloride passes into the fish until the normal salt content is regained.

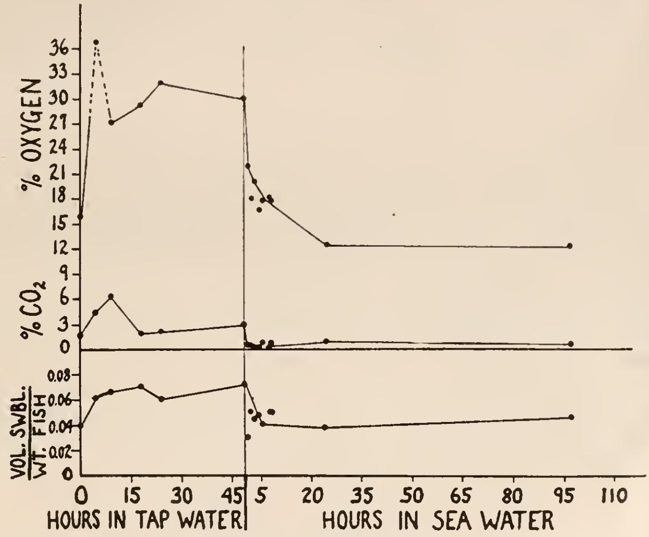

FIGURE 4. Changes in swimbladder gas of *Fundulus heteroclitus* transferred from sea water to soft tap water for 2 days, then returned to sea water.

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On the other hand, the gills may not be equally permeable in both directions; or the fish may have some means of controlling movement of water and salts by neural and hormonal activity in a manner similar to that described for the adrenal cortex of dogs (Loeb *et al.*, 1933) and pituitary of frogs (Boyd and Whyte, 1938).

The difference between the time required for the separation of gases from the blood, to increase the gas volume in the swimbladder in fresh water, and that necessary to resorb the gases on returning to sea water might be explained by assuming that the active secretion of gases into the swimbladder would demand more time and energy than the passive resorption of the gases by the blood.

When experiments of the type described in this paper are applied to fish in various stages of acclimatization, a graphic description of the process would then be available which might facilitate further work on the mechanisms involved in acclimatization to salinity changes.

SUMMARY

1. The adjustment of the killifish, *Fundulus heteroclitus*, to fresh water involves significant changes in the swimbladder gas, chloride content, weight, and density of the fish. Adaptation to fresh water is complete after 24 hours.

2. The gain in weight is only temporary, returning to normal after 18 hours The gain is due to taking on water from the hypotonic medium. The subsequent loss may be the result of kidney excretion and inanition.

3. The chloride decreases markedly during the first 12 hours but some appears to be regained after 24 hours. This irregularity corresponds to the short period of weight gain and loss, and appears because the water taken on by the fish is included in the calculation of chloride on the basis of weight. After 4 days in fresh water the fish have lost approximately 60 per cent of their normal chloride content.

4. When the fish are first put into fresh water they sink immediately to the bottom because fresh water is less buoyant than sea water. In order to adjust to the change and regain normal buoyancy the fish deposit oxygen and, to a lesser degree, carbon dioxide into the swimbladder. The volume of gas measured at barometric pressure is greater after adjustment to fresh water, showing that the amount of gas in the swimbladder has increased.

5. All the above adjustments tend to decrease the density of the fish to approximately the density of the water within 24 hours.

6. When fish are replaced in sea water after two days in fresh water, they regain their normal chloride, density, and swimbladder gas within six hours, or four times as fast as the previous adjustment to fresh water. Possible reasons for this difference in rate of adjustment are discussed.

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A NEW METHOD OF REPRODUCTION IN OBELIA

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In view of the eminence of *Obelia* as a zoological type enthroned in all text books, and the consequent widespread study of innumerable specimens, it is surprising that there could be an important method of reproduction of this genus so far unreported.

The observations recorded here were made in the course of extensive investigation of growth and form in *Obelia,* involving day by day study of specific colony sites through the summer of 1947 at Boothbay Harbor. Temperature changes were followed closely since colonies of *Obelia* and other hydroids fluctuated greatly, disappearing and reappearing as temperatures rose and fell markedly above and below 20° C. Three species were studied, all associated with one float, *Obelia articulata, O. geniculata* and *O. longissimus.*

For nearly two months of excessively high temperatures during July and August, no colonies could be found. With the onset of offshore winds, the warm surface water blew out of the bay, to be replaced by bottom water 8 to 10 degrees colder. With this lowering of the temperature, small *Obelia* colonies appeared in relatively large numbers. Calm weather with no wind except the daily inshore breeze allowed the surface bay waters to warm up again to about 21° C. for a few days, followed by a slow fall to lower temperatures. The growth or reproductive procedures described here were responses to these changes.

OBELIA ARTICULATA

This species grew attached to laminaria. Colonies are relatively small but well branched, and in general are intermediate in character between the single unbranched stems of *O. geniculata* and the enormously long and branching colonies of *O. longissimus.* The intermediate character is again shown in the distribution of the gonangia. In *O. geniculata* they grow out from the angles made by the hydranths and the stem. In *O. longissimus* they appear at the angles made by hydranths with lateral branches but only at the basal region of a colony after it has already become massive. In *O. articulata* they appear when the colony is small, but at angles between hydranths and secondary branches, not in connection with the main stem. Similarly the growing tip of the main stem in colonies of all three species varies in series. It is essentially a stolonic type of growth like that of the creeping stolons. In *O. longissimus* it grows rapidly and vigorously, giving off secondary stolonic outgrowths regularly at a certain distance from the tip, and these behave in much the same way. Hydranths are mainly tertiary outgrowths, at least. In *O. geniculata* terminal stolonic growth is very limited, lateral branches are not formed, and the tip itself usually differentiates into a hydranth. *O. articulata* lies between.

In any species a rise in temperature, especially when in excess of 20° C., tends to maintain or promote stolonic growth at the end of a stem or branch of any order and conversely to inhibit hydranth differentiation. The outgrowths capable of responding in one way or another to different temperature conditions may be of varying origin. They may be the terminal tips of the main stem and secondary branches, tips of branches of a more subsidiary order, or the tips of presumptive gonangia at stem or branch angles.

A marked rise in temperature results in prolonged growth of a stolonic character in the first two cases, the long slender branches thus formed remaining an integral part of the colony, even though they may in no way contribute to its welfare. In the third case, those normally destined to become gonangia, the reaction is different.

A gonangium in its earliest recognizable stage is shown in Figure 1A, growing from the non-annulated region immediately below a hydranth. It consists of an outgrowth with several annulations, terminating in a relatively large bulb with a short central cone and wide shoulders. This persists and grows as the distal cap of the gonangium. In the same figure on the same scale are shown outgrowths from homologous locations, but from colonies subject to higher temperatures. Annulated growth, instead of stopping after two or three annulations and forming the wide gonangium rudiment, continues until ten or a dozen shallow annulations have occurred. The final surge corresponding to the establishment of the gonangium leads instead to the formation of a massive elongate stolonic structure with no further trace of annulations. It is similar to the terminal stolonic growth at the ends of branches, but with two differences, it is much more massive and of greater girth, and is so vigorous that the stem uniting it to its point of origin becomes attenuated to the point of rupture (Fig. 1B). Distally each such mass grows rapidly, while it resorbs correspondingly at the proximal end. The separation usually occurs at the region where the annulated growth transforms into a steady surge (Fig. 1C), the part left attached to the colony retracting proximally as the tension is relaxed, while the congested terminal units slide out of the thin but wide perisarcal tube to float freely in the surrounding water.

The question that arises at once is whether this is a normal process or a response to the disturbance of collection and subsequent examination. Obelia and similar hydroids are notoriously susceptible and it is a common experience to find hydranths and other terminals in process of regression with distal parts of the coenosarc often isolated within the perisarc from the main body. This possibility was considered immediately, and the following is the evidence that the process is a normal one for the sea temperature prevailing at the time. Colonies picked off the laminaria and dropped into formalin within a few seconds of emergence from the water exhibited the phenomenon to as great a degree as any. The colonies under live examination were fresh, had hydranths with active tentacles and manubrium, possessed hydranth buds that progressed normally to complete development, and showed no trace of resorption at any of the terminals.

Small colonies left standing in finger bowls liberated literally hundreds of gonangial terminals overnight and were still in active process the following day. Lastly, there is the evidence that they possess a useful function. In the first place, fragments of ordinary terminals of equivalent length but smaller diameter can reattach and survive for a week or two. They do not develop hydranths unless

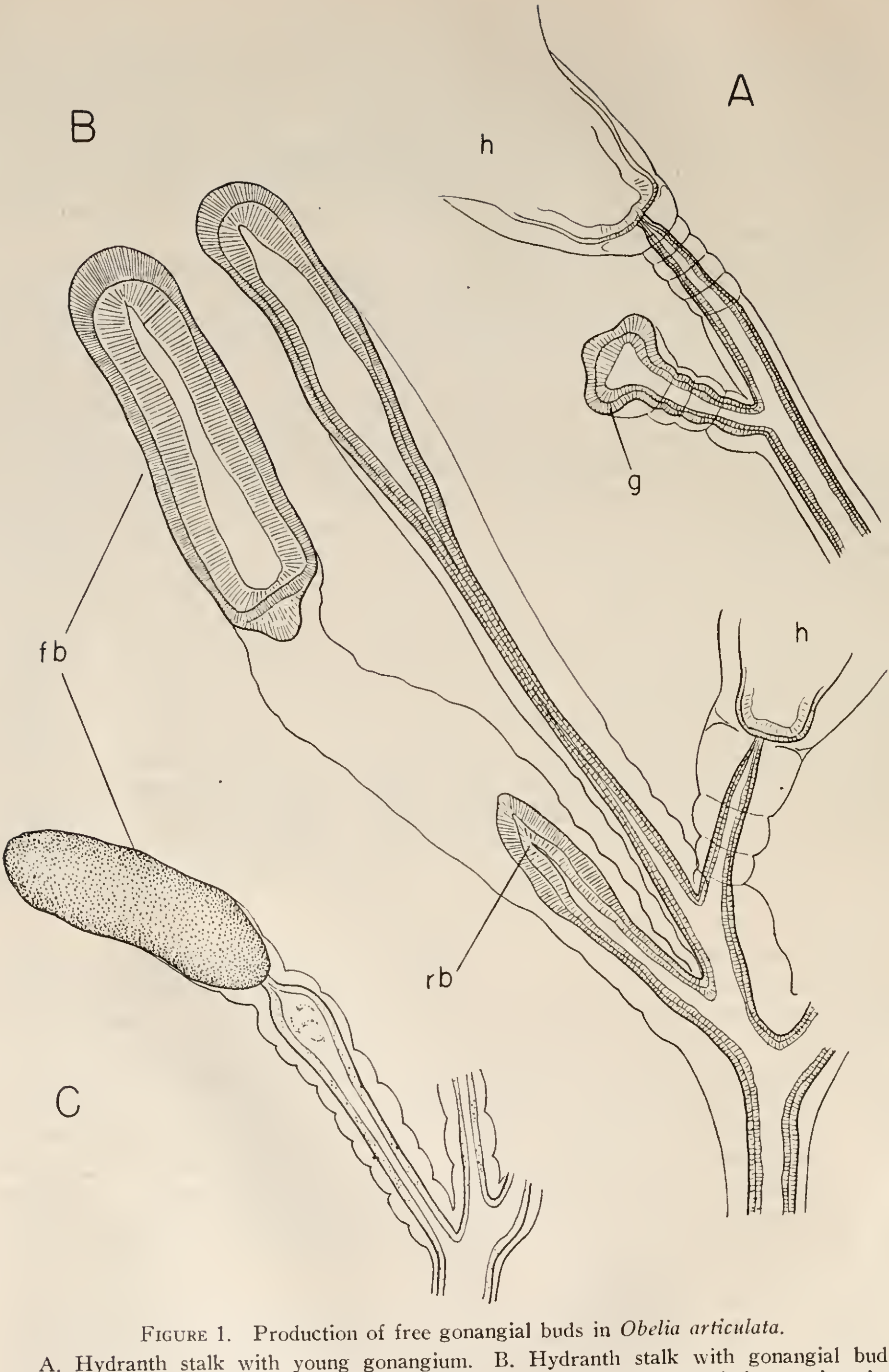

FIGURE 1. Production of free gonangial buds in *Obelia articulata.*

A. Hydranth stalk with young gonangium. B. Hydranth stalk with gonangial buds in process of formation and liberation. C. Gonangial bud showing constriction at junction of annulated and non-annulated regions. *fb,* free buds; *g,* young gonangium; *h,* hydranth; *rb,* retracted stalk after liberation of bud.

several times as long. The isolated gonangial terminals on the other hand become attached to a solid substratum immediately upon contact. After about 12 hours, each fragment is about twice its original length and half its girth. The original perisarc, however, indicates that most of the tissue is now the result of new distal growth and proximal resorption (Fig. 2B). At the same time an annulated up-

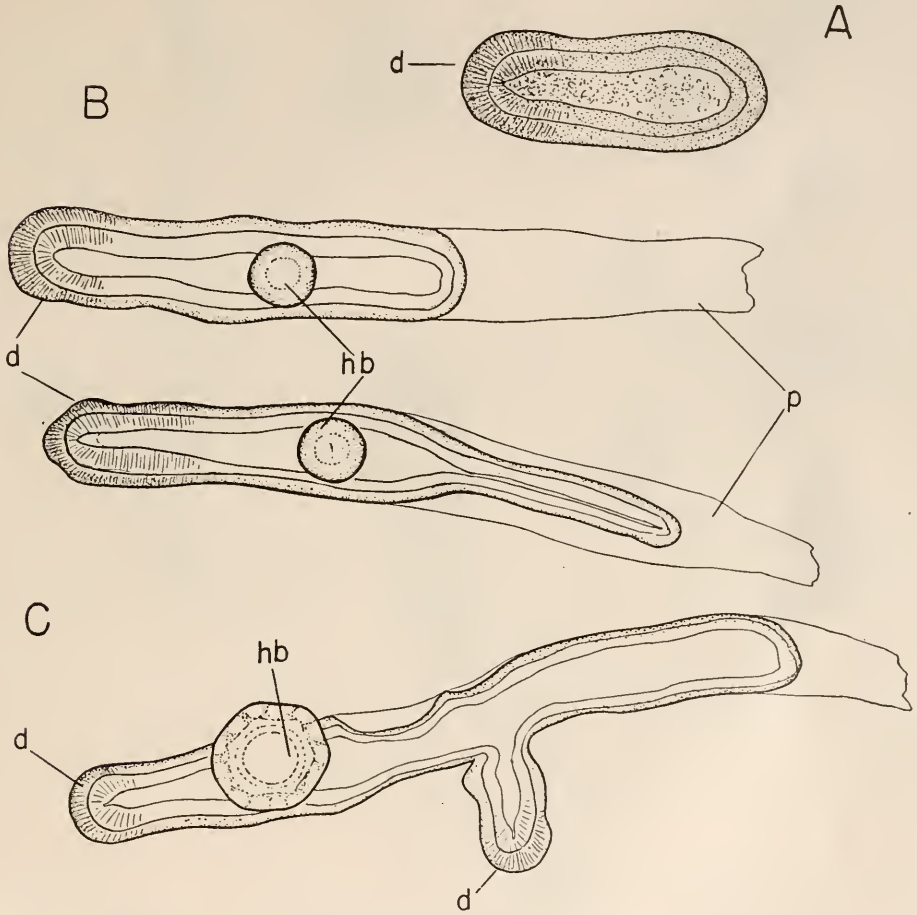

FIGURE 2. Development of free gonangial bud of *Obelia articulata*.

A. Bud at time of attachment. B. Twelve hours later with hydranth buds growing vertically and empty perisarc indicating extent of proximal resorption and distal growth. C. Twenty-four hours after attachment, with hydranth bud at tentacle rudiment stage, and with secondary distal outgrowth. *d,* distal growing region; *hb,* hydranth bud.

growth from the middle of the fragment indicates a developing hydranth. In Figure 2C a fragment is shown typical of the condition 24 hours after liberation. A lateral creeping stolonic terminal has started, while the hydranth has progressed to the tentacle rudiment stage. In the great majority of the liberated fragments, functional hydranths were present on the second day and new colonies thus started.

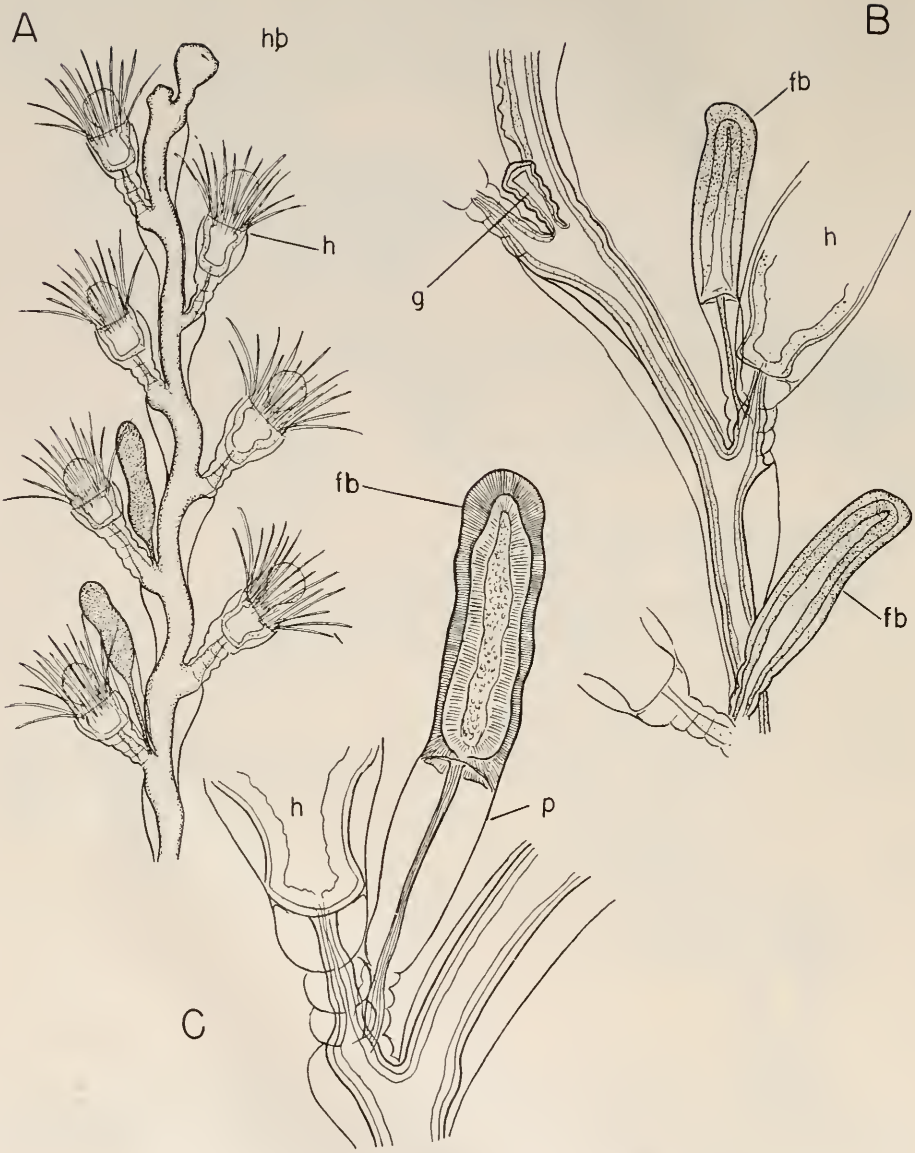

FIGURE 3. Production of gonangial buds in *Obelia geniculata.*

A. Complete sprig with two advanced gonangial buds. B. Part of stem showing young gonangium and two stages in production of gonangial buds. C. Bud showing sharp demarkation between massive presumptive free bud and attenuated proximal stalk. *fb,* presumptive free bud; *g,* gonangium; *h,* hydranth; *p,* perisarc.

The immediate developmental capacities are accordingly somewhat superior to those of the average planula.

Obelia geniculata

Colonies of *Obelia geniculata* were collected a day later than those of *O. articulata*, when the water temperature was already falling. The great majority of stems had the appearance shown in Figure 3A. No gonangia were present, but in their place were large congested terminals similar to those of *O. articulata*. Detail as seen in Figure 3C indicates that the process is essentially the same, the distal part of the massive stolonic outgrowth growing rapidly at the expense of proximal tissue. In fact the proximal half of the outgrowth becomes so attenuated that the lumen is obliterated. While actual separation was not observed in this species, continued distal growth after the occlusion of the lumen must inevitably result in a break in the attenuated proximal part. In Figure 3B two stages are shown, one with an attenuated stalk and a younger stage with wide lumen throughout. A third, the most anterior, is a younger outgrowth and is developing into a typical gonangium, suggesting that the external temperature had already dropped below the critical value at the time of its initiation. At the same time it indicates the relative scale of the two forms of growth from the hydranth-stem angles, and the comparative massiveness of the high-temperatures' outgrowth.

Obelia longissima

This species is included merely as a basis for comparison. It is typified by the very extensive growth of the primary and secondary terminals, leading to the formation of relatively enormous colonies. Gonangia appear in secondary and other angles at the base of the colony only after it has attained a fairly large size. During the warmer summer months growth is directed mainly into the vigorous terminals, and there appears to be little tendency to form gonangial outgrowths at all. They are most abundant during late winter and early spring. Consequently the type of asexual reproduction just described for *O. articulata* and *O. geniculata* is here probably of very rare occurrence, if it occurs at all, for without the initiation of gonangial outgrowths of any kind, no response in either direction is possible.

Summary

A method of asexual reproduction previously unrecorded is described for *Obelia articulata* and *Obelia geniculata*.

When water temperatures markedly exceed about 20° C. presumptive gonangial outgrowths continue growth as massive stolonic terminals that rapidly constrict off, leave the colony and settle elsewhere to establish new colonies in large numbers. In size and potentiality these reproductive units somewhat exceed those of typical campanulid planulae.

TOOTH SUCCESSION IN THE SMOOTH DOGFISH, MUSTELUS CANIS

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AND

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The arrangement of the teeth of sharks in a series of rows is well known. In some species, such as tiger sharks and sand sharks, with large conical teeth, newly formed teeth appear to be formed in the back rows while older teeth are in front. This impression led Owen in 1866 to state, ". . . the whole phalanx of their numerous teeth is ever marching slowly forwards in rotary progress over the alveolar border of the jaw, the teeth being successively cast off as they reach the outer margin, and new teeth rising from the mucous membrane behind the rear rank of the phalanx." Owen's theory of tooth replacement in sharks is the commonly accepted one today and is found in most comparative anatomy texts. This theory apparently was based only on morphological evidence without experimental proof; a search of the literature has failed to reveal reports of any experiments testing the theory. However, the morphological evidence is quite convincing and accounts for the general acceptance of the theory.

Within recent years Owen's hypothesis has been challenged by Cawston in a series of papers (1939; 1940a, b, c; 1941a, b, c; 1944; 1945). He has doubted that sharks shed their teeth but if they do he denies the possibility of replacement occurring by the forward movement of teeth from the rear. That sharks shed their teeth is confirmed by Breder (1942) who noticed the sloughing of teeth by sand sharks (*Carcharias littoralis*) in the tanks at the New York Aquarium. Whether the lost teeth are replaced and the manner of this replacement if it occurs apparently has not been observed. It is the purpose of this investigation to inquire experimentally into the question of polyphyodonty in selachians.

MATERIALS AND METHODS

It was thought at the beginning of this work at Woods Hole, Massachusetts, that both the spiny dogfish (*Squalus acanthias*), and the smooth dogfish (*Mustelus canis*) could be used. However, the spiny dogfish would not live in the aquaria. Perhaps this may be caused by normal summer salt water temperature in Woods Hole being lethal for the spiny dogfish but not for the smooth dogfish. This was suggested by William Schroeder, Jr., of the Woods Hole Oceanographic Institute who in conversation with the authors pointed out the coastwise migrations of the spiny dogfish paralleling temperature isotherms.

Since *Squalus* proved unsatisfactory, *Mustelus canis*, collected at Woods Hole, Massachusetts, were used in these experiments. A total of 23 adult animals were used, one group of 12 in the summer of 1946 and a second group of 11 in the summer of 1947. The animals ranged in size from 14½″ to 39″ with the majority being over 24″ in length; 11 were males, 12 females. They were kept in a large paraffin-lined cement tank supplied with running sea water and were fed every other day on chopped fish.

The dogfish were anesthetized by cooling in ice water according to the method of Parker (1937) and a varying number of teeth, as described below, were removed with forceps from the lower jaws. In order to follow the movements of the remaining teeth they were marked with silver nitrate solution precipitated with stannous chloride. While the stain subsequently was worn away from the surface of the teeth, sufficient amounts remained on the sides of the teeth to mark them adequately. This species has pavement teeth, somewhat diamond-shaped and arranged in compact rows (see Fig. 3). Sections were made of the jaws using both paraffin and celloidin technics following decalcification. Mallory's stain as well as haemotoxylin and borax-carmine was used.

We wish to thank the Woods Hole Oceanographic Institute and the Marine Biological Laboratory for the use of their facilities.

Experiments and Observations

The preliminary experiments were designed to determine if tooth replacement occurs in *Mustelus*. For this purpose 12 animals were divided into four groups. In the first group of three animals, six teeth of the first row in the mid-line of the lower jaw were removed. These animals died six, eight, and 11 days respectively after the operation. The cause of death was not ascertained although it probably was not the result of the operation since one of the unoperated controls died during the same period. The teeth were not replaced in this period. Serial sagittal sections at 10 μ revealed no change had taken place and the jaws presented the usual appearance with tooth buds in successive stages of development posterior to the area of the erupted teeth.

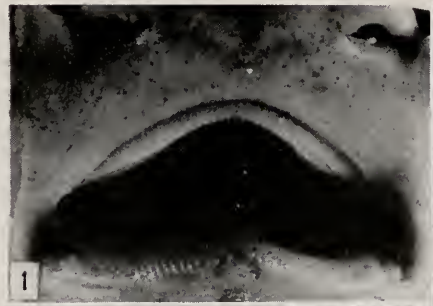
The second group contained four animals from each of which 22 teeth were extracted from a triangular area, five rows deep; the apex of the triangle pointed posteriorly. Figure 1 is a photograph of a jaw of this group. Two of the fish died before replacement occurred, after eight and 12 days respectively. The remaining two replaced the teeth within 50 days. Figures 2 and 3 are photographs of the jaw of one of these latter fish. It can be seen that the replaced teeth are arranged in the normal pattern. Sections of these jaws also were normal in appearance (Fig. 4).

The third group of three animals had the first row of teeth removed. Two died on the following day but the third had replaced the teeth when examined 93 days later. The rate of replacement was not obtained for this animal.

The fourth group consisted of the two control animals. Both were anesthetized by cooling but were not operated upon. One died the following day, the other in 18 days. The cause of death was not determined although the method of anesthetizing might have been a contributory cause.

The second series of experiments were designed to discover the manner in which the tooth replacement occurred. The 11 dogfish of this series were divided into three groups. In the first group of four, each of the fish had 12 teeth in all ex-

Plate I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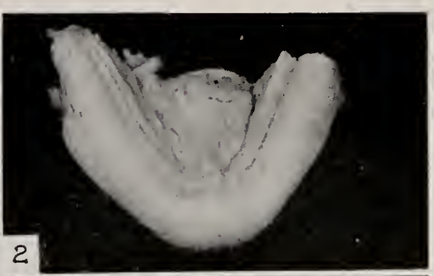
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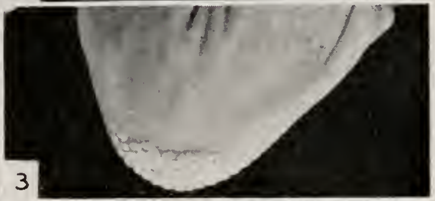
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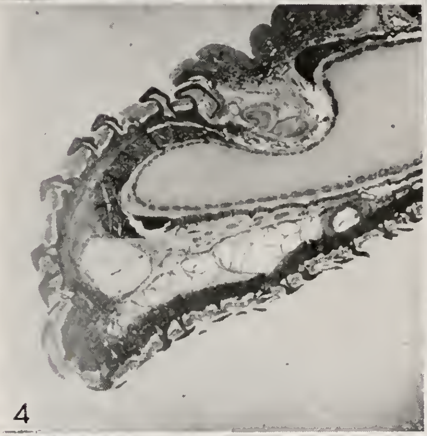
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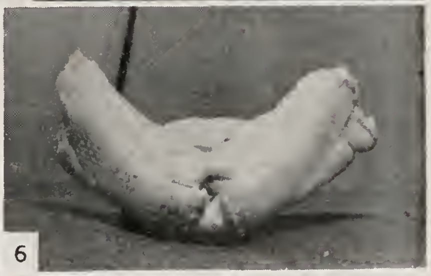
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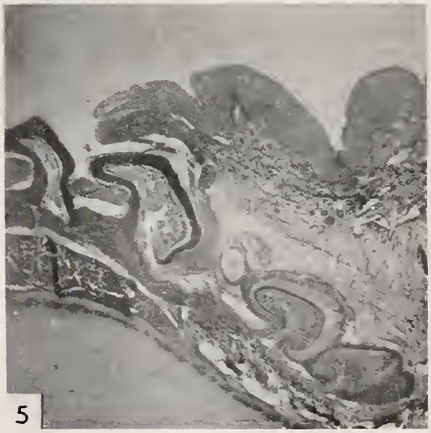
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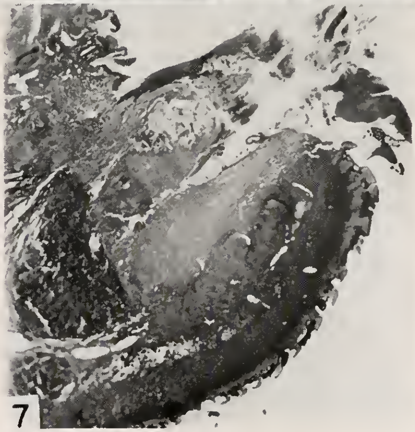
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tracted from the anterior first two rows in the center section of the lower jaw. The remainder of the teeth with the exception of the two first rows lateral to the extracted area were marked with silver nitrate. One animal died on the ninth day and no change in the teeth was found. The other three were examined 25 days later and all had replaced the extracted teeth with teeth bearing silver nitrate marks. In addition, the teeth lateral to the extracted area, previously unmarked, now were replaced by teeth bearing silver nitrate markings. This would seem to indicate, therefore, that within the 25-day period, two rows of teeth moved forward and replaced the former first two rows.

The second group of this series consisted of five animals in which either two, three, or four rows in the center section were removed, and the tooth-bud area back of the region from which the teeth had been extracted, was cauterized with an electric cautery. Four of these animals died in three, five, 12 and 13 days respectively. The remaining animal of the group lived and was killed 25 days later. In the three cauterized dogfish living 12, 13, and 25 days the tooth area in front of the region cauterized was disorganized: many teeth in addition to those extracted had fallen out and only a few scattered teeth remained in the center area. Figure 6 is a photograph of the jaw of one of these fish. No replacement of teeth had occurred in any of this group including the animal killed after 25 days. A section (Fig. 7) from this latter dogfish taken through the cauterized area and the region anterior to it shows the drastic disorganization resulting from the cauterization. The tooth buds were destroyed and parts of the jaw cartilage degenerated. The oral epithelium and underlying connective tissue appeared to be sloughing off.

The third group contained two animals in which all but the first two rows of teeth were marked with silver nitrate but no teeth were extracted. Both of these fish died six days later; there were no observable changes in the teeth.

Certain general observations of the teeth were made. It was found that the first or outermost row of teeth was irregular while the preceding rows are quite regular. This would seem to indicate that the teeth are normally lost singly from the first row as has been observed in other species. Great regularity was observed in the posterior rows and in the animals examined there were no indications of tooth-loss except in the first row. The number of exposed rows of teeth varied from eight to 11. No sexual differences in the teeth were seen. The arrangement of the teeth in the upper jaws appeared to be similar to that of the lower jaws.

PLATE I

FIGURE 1. View of jaws of dogfish showing triangular area in center of lower jaw from which teeth have been extracted. About one-third natural size.

FIGURE 2. Dorsal view of jaw of animal in Figure 1 fifty days after removal of teeth showing the complete replacement of the teeth. About one-third natural size.

FIGURE 3. Ventral view of jaw in Figure 2. About one-third natural size.

FIGURE 4. A sagittal section at 10 microns of the jaw seen in Figure 2. Tooth buds can be seen back of the erupted teeth. About × 10.

FIGURE 5. A view of the tooth bud area from Figure 4. About × 33.

FIGURE 6. A dorsal view of a jaw in which 4 rows of teeth were removed in the center section and the tooth buds back of this region were cauterized. No replacement had occurred after 25 days. About one-third natural size.

FIGURE 7. A sagittal section at 10 microns of the jaw seen in Figure 6, showing the disorganization resulting from the cauterization. About × 10.

Tooth-bud areas were never found except behind the tooth-bearing region. Figure 5 is a photograph of the tooth-bud area. The tooth buds can be seen to be progressively larger and more mature in a postero-anterior direction. Particular care was taken to search for buds underlying the outermost rows but none were found. It would appear, therefore, that the only source of new teeth are these buds back of the erupted tooth area.

Conclusions and Discussions

From the experiments described above it seems apparent that in *Mustelus canis* teeth can be replaced and that this replacement occurs in the manner hypothecated by Owen; that is, by the moving forward of the teeth from the rear. The fact that marked teeth from posterior areas were seen later to occupy areas where teeth had been removed seems conclusive evidence in favor of Owen's view. It is not certain from the experiments what the normal rate of replacement is since the animals which were to have been used to test this point died before such information could be obtained. However, the rate of replacement in the operated animals was quite rapid, being approximately of the order of one row replaced in ten to twelve days.

The experiment in which the tooth buds back of the center area of the jaw were removed by cautery was done to determine whether replacement occurred in the absence of the posterior tooth buds. In the one surviving animal replacement had not taken place although in the same length of time non-cauterized dogfish did replace teeth. While the experiment apparently bears out the role of the posterior tooth buds in replacement it may be criticized on the ground that the unexpected general disorganization and degeneration resulting from the cauterization would prevent replacement from any source. However, even if this experiment is omitted from consideration, there is sufficient evidence from the other experiments to support the contention that Owen's hypothesis is correct.

From a study of Cawston's papers it would appear that his views are based on gross examination only and without a study of histological sections. Otherwise it is difficult to account for his statement (1941a): "New tooth formation behind the normal number of rows of teeth in species of shark has never been observed, though dental germs should be present if the alleged replacement of teeth by revolving of the gum forwards ever occurred in adult specimens." In the same paper he also states: "At the anterior border of the teeth of *Mustelus canis* (Mitch.) one sees round or oval dental germs in process of development into the flattened closely set teeth of the adult, which reveal the characteristic wrinkled surface very early." As we have noted earlier, and as can be seen from the photographs of the sections (Figs. 4, 5), tooth buds are found back of the erupted teeth and are not found in the front region of the jaw. There is no evidence that new teeth are being formed in the front row of *Mustelus*.

In a later paper (1944), Cawston states that there is no provision for replacement of lost teeth in selachians and that growth may continue throughout life. In earlier papers (1939, 1941a) he considers that a tooth is renewed at the site where one is lost. He considers that this replacement obtains by vertical succession (1941b). Unless we are misinterpreting the statements it would appear that Cawston's viewpoint has changed from a possibility of vertical succession in tooth replacement to the hypothesis that no replacement of any type occurs.

Other observers besides Owen have concluded by studying the morphology of the jaw that replacement occurs by the forward movement of the back teeth. For example, Budker (1938) states: "Lorsque la dent est tombée, une autre, dite 'dent de remplacement' et provenant des rangées de remplacement disposées derriere les rangées fonctionelles, vient prendre sa place." This author also observed that tooth buds did not develop at the site of the lost tooth.

The cause of the falling-out of the teeth was also studied by Budker in various species such as *Scyliorhinus canicula.* He accounted for this loss by the destruction of the dentinal basal plates which anchor the tooth in the underlying connective tissue by specialized cells similar to osteoclasts which cells also reduce the dentine of the older tooth as a whole. Benzer (1944), on the other hand, reports that the dentine of *Mustelus* grows progressively thicker in older teeth. He did not note that the dentine was later destroyed.

The jaws of ten other species of sharks were examined by the authors through the courtesy of Mr. Schroeder at the Museum of Comparative Zoology at Harvard University. Included in the group were three species of the Port Jackson shark (*Cestracion* or *Heterodontus*) which have pointed biting teeth in front and flat crushing teeth in the remainder of the jaw. It was observed, however, that the teeth in any section of the jaw are the same in an antero-posterior direction and consequently could be replaced in the manner described for *Mustelus.* No morphological indications were found in any of the other species examined contradicting Owen's hypothesis.

SUMMARY

1. Twenty-two teeth extracted in a triangular area five rows deep from the front of the tooth-bearing region of the lower jaw of *Mustelus canis* were replaced within 50 days.

2. Marking of the posterior teeth with silver nitrate indicated that extracted teeth were replaced from behind by these marked teeth. The replacement rate was approximately one row in 10 to 12 days.

3. Tooth buds were found only back of the erupted teeth and never elsewhere.

4. Destruction of the tooth buds by cautery prevented replacement.

5. It is concluded that Owen's hypothesis of the replacement of sharks' teeth by the forward movement of the posterior teeth is correct and that Cawston's objections to the theory are not tenable.

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POSTEMBRYONIC GROWTH CHANGES IN THE ISOPOD PENTIDOTEA RESECATA (STIMPSON) WITH REMARKS ON THEIR TAXONOMIC SIGNIFICANCE

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Introduction

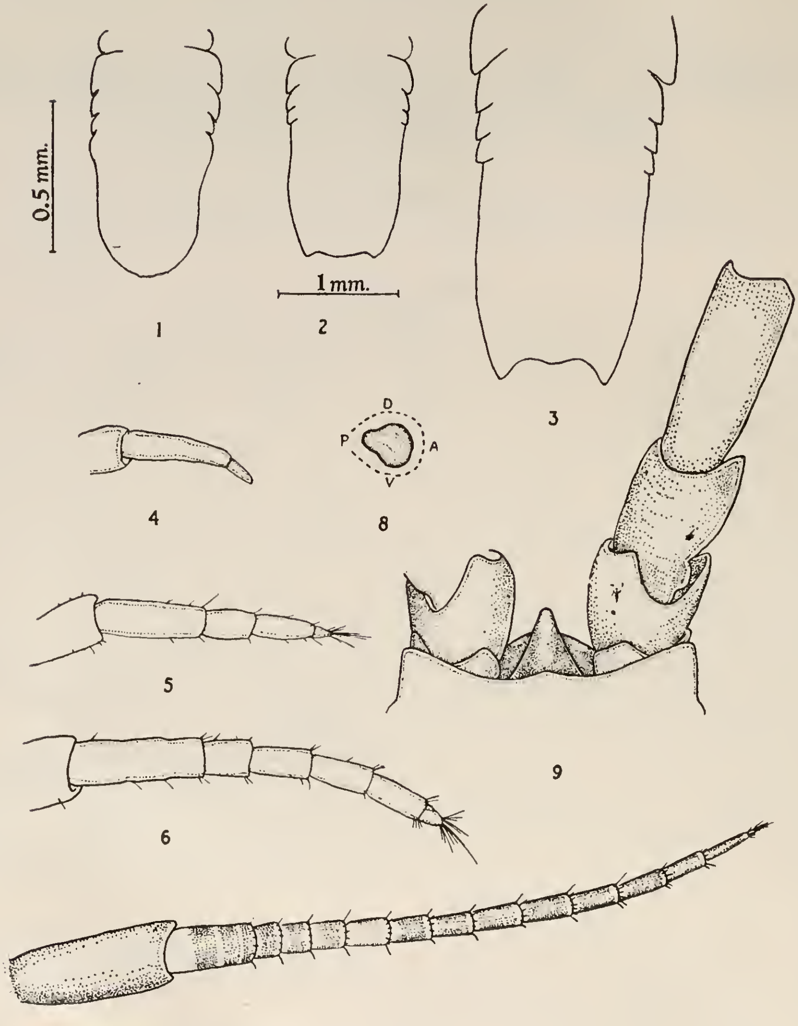
This study was the result of the observation that the number of setae of the seventh peraeopod of *Pentidotea resecata* (Stimpson) (Valvifera: Idotheidae) was markedly variable. It was decided to conduct an investigation including features other than peraeopod setal number, in order to determine which features remained relatively stable and were thus of specific significance in the classification of the marine idotheids. The nature of the variations in certain features was found to be directly related to the size of the specimens, and thus to growth; and it is believed that these variations are of basic significance to isopod taxonomy. The features at present used to distinguish marine isopods of North America of the family Idotheidae include the number of segments of the flagellum of the second antennae, the number of segments of the palp of the maxilliped, and the shape of the posterior margin of the telson (Richardson, 1905 and references, pp. 346–408). It is most significant that, in the species investigated, it was these features that demonstrated the greatest degree of growth variation.

The material consisted of ten adult and seven juvenile specimens which ranged in length from 5.2 mm. to 43.0 mm. and of ten far advanced embryos of 2.2 mm. length, removed from the marsupium of an adult female of the species. The specimens were collected by the writers during the summer of 1947 from eelgrass, *Zostera* sp., located on the sand flats of Tomales Bay, Marin County, California, where the species is fairly abundant.

The head. The most stable features of the head during growth included the shape and location of the eyes (Fig. 8) and also the relationship of the frontal laminae to one another and to the anterior-dorsal border of the head. In embryos, however, these features were not developed.

The number of segments to the flagellum of the second antennae was found to increase in direct proportion to the size of the specimen at least until adult status was reached. This phenomenon was observed in part by Hale (1946, Fig. 19, p. 193) in his description of *Antarcturus horridus* Tattersall (Arcturidae). Embryos 2.2 mm. long had two segments to the flagellum of the second antenna. In a specimen of 5.2 mm. and one of 6.0 mm. length the number of segments was four. In two specimens of 8 mm. length, one of 9 mm. length and in one specimen of 10 mm. length the number of segments was seven; while in one specimen of 9.5 mm. length the number of segments was eight. Even among the larger specimens the number of segments of the flagellum was observed to vary considerably. The length of the flagellum appears to be proportional to the body length in the juvenile

Plate I



Pentidotea resecata

Figure 1. Abdomen, dorsal view, embryo, 2.2 mm. length. Magnification as indicated.

Figure 2. Abdomen, dorsal view, juvenile, 5.2 mm. length. Magnification as indicated.

Figure 3. Abdomen, dorsal view, small adult male, 9.5 mm. length. Magnification as per Figure 2.

Figure 4. Flagellum second antenna, left, embryo, 2.2 mm. length. Magnification as per Figure 1.

Figure 5. Flagellum second antenna, left, juvenile, 5.2 mm. length. Magnification as per Figure 1.

Figure 6. Flagellum second antenna, left, small adult male, 9.5 mm. length. Magnification as per Figure 1.

specimens of *P. resecata,* while in the adult specimens the length of the flagellum varied.

In specimens of 6.5 mm. and below, the palp of the maxilliped was four jointed. More developed specimens had a maxilliped palp of five segments. At present the only character used to distinguish the genus *Pentidotea* (Richardson, 1905, p. 368) from *Idothea* (ibid., p. 356) is the presence of a five-jointed palp in the former and of a four-jointed palp in the latter (see also Light, 1941, p. 87). It seems evident to the writers that either *Pentidotea* must be considered a synonym of *Idothea* or that the generic differences must be redefined.

The increase in setae on the tip of the endopodite of the maxilliped was found to be correlated directly with the size of the specimen (compare Fig. 17 and Fig. 19). The same was true of the "hairiness" of the median border of the palp of the maxilliped. Only the presence of a single coupling-hook on the median border of each maxilliped was found to be constant (Figs. 17 and 19, "x").

The perion. The relationship in length of the lateral border of the epimeral segments of the perion to the length of the lateral border of the perion segments themselves appeared to be constant, yet measurements made at the second and third perion segments showed considerable variation. This variation did not correlate directly with a size increase of the specimens and the writers believe that the difficulty in obtaining accurate measurements of these structures accounts for the irregularity. Observations indicate that the seventh perion segment remained in an undeveloped state in juvenile animals as large as 5.2 mm. Its retarded development was best indicated on embryo specimens. The general narrow shape of the animal was maintained in animals of all sizes. Ovigerous specimens showed a distinct lateral widening of the segments of the perion concerned with the marsupium development.

The peraeopod. The number of setae on the seventh peraeopod was examined carefully in the hope that a very definite non-variable structural feature could be found. The number of setae on the ventral surface of the propodus was observed to be directly proportional to the size of the animal. Three distinct types of setae are discernible; a "saw-toothed" seta (Fig. 11), a "file-toothed" seta (Fig. 10), and a "simple" seta (Fig. 12). A once specialized seta observed on the propodus of the seventh peraeopod of a small animal was always found without modification, other than increase in size, on the propodus of the seventh peraeopod of a larger animal. The "saw-toothed" type seta was constant in number and location. The "file-toothed" type seta was more numerous on the propodus of the seventh peraeopod of larger specimens and the same is true of the "simple" type setae which most frequently surrounded the "file-toothed" seta. Evidence indicates that the "file-toothed" seta is developed, in part at least, from one of the "simple" type.

It was found that the seventh peraeopod was weakly developed in very small specimens (below 6.0 mm. length); whereas the first to the sixth peraeopods were

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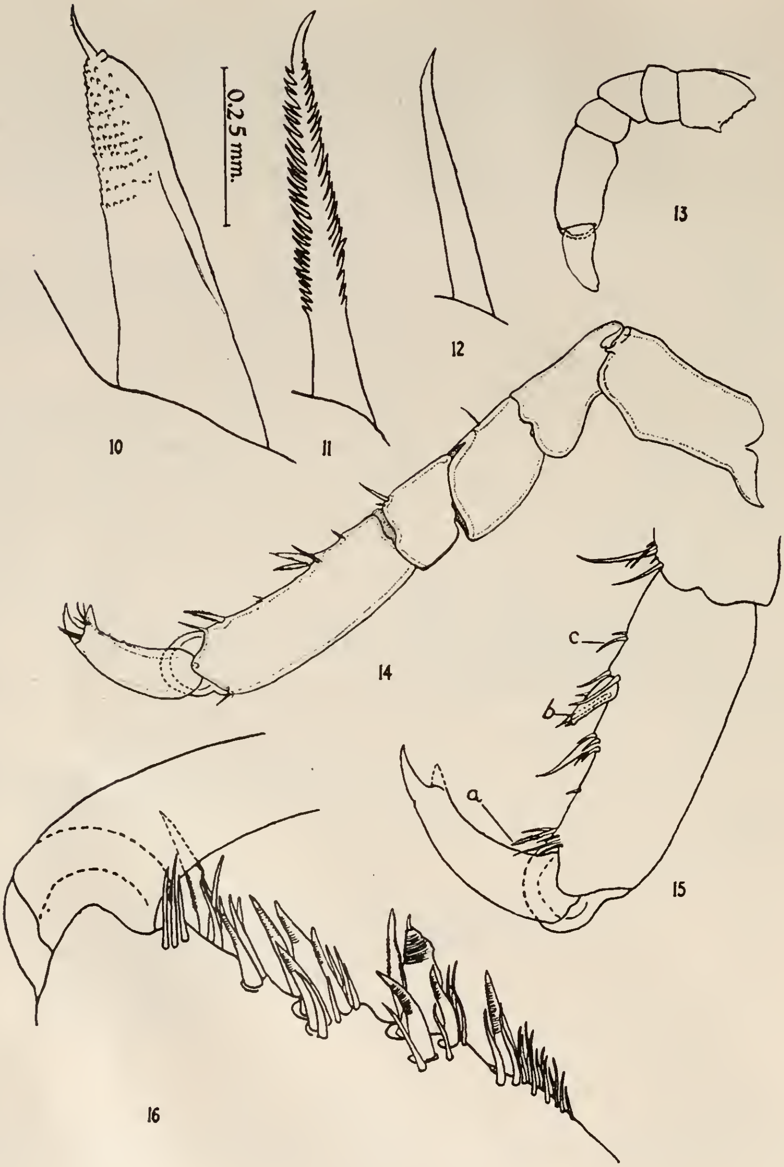
FIGURE 7. Flagellum second antenna, left, adult male, 20.5 mm. length. Magnification as per Figure 2.

FIGURE 8. Eye, left, lateral view, adult male, 20.5 mm. length; "P" posterior, "A" anterior, "D" dorsal, "V" ventral. Magnification as per Figure 2.

FIGURE 9. Anterior-dorsal border and first two frontal laminae, adult male, 20.5 mm. length. Magnification as per Figure 2.

PLATE II



Pentidotea resecata

FIGURE 10. File-toothed seta of ventral surface of propodus, see "b" of Figure 15. Magnification as indicated.

FIGURE 11. Saw-toothed seta of ventral surface of propodus, see "a" of Figure 15. Magnification as per Figure 10.

FIGURE 12. Simple seta of ventral surface of propodus, see "c" of Figure 15. Magnification as per Figure 10.

well developed. The retardation in the development of the seventh peraeopod has been observed in other isopods (Sømme, 1940, Limnoriidae, p. 158; Faxon, 1882, Asellidae, pl. vi, Fig. 19; Hult, 1941, Parasellidae, p. 39).

The telson. The concavity of the posterior margin of the telson has been regarded as the most diagnostic and key feature of this species. Actually, however, the margin was found by the writers to change very gradually from one with a convex posterior border in the embryo (Fig. 1) to a slightly concave margin in specimens of 6.5 mm. in length (Fig. 2); until, when the adult condition is reached (Fig. 20), the concavity is most developed.

The uropod conforms to the shape of the telson and therefore varies in accordance with its size and shape. The number of movable setae at the lateral articular distal border of the penultimate uropod segment varied in number from one to two regardless of size or sex of the specimen.

It is evident from the above observations that very young specimens of *Pentidotea resecata* might very well be placed in the genus *Idothea* and considered new to science by an investigator unaware of the developmental nature of the maxilliped palp. Such would be true at least as long as the two genera *Idothea* and *Pentidotea* remain so briefly designated. Indeed one writer, Fee (1926, p. 18, Fig. 12) did just that in describing *Idothea rufescens* from specimens which apparently are juvenile specimens of *Pentidotea resecata* (Stimpson).

The suggestion of course from the above is that authors of new species of Idotheid genera (as well as isopods in general) not only indicate the measurements of the types but also give measurements of all specimens figured or described in the text. To date such a procedure has been followed by only a very limited number of workers and even then without any marked degree of consistency.

SUMMARY

In an attempt to find constant characteristics which may be relied upon as specifically diagnostic in the marine isopod *Pentidotea resecata,* the following features proved to be especially significant constant features regardless of the size of the specimen: (1) structural interrelationships of the frontal laminae, (2) epimeral plate length in relation to the length of the lateral border of the corresponding perion segment, (3) the character of the setation and certain features of peraeopod morphology, (4) general body shape.

Features showing numerical increase which was found to be directly proportional to the size of the animal and thus believed to be of very limited taxonomic utility include: (1) number of segments to the flagellum of the second antennae, (2) number of segments to the palp of the maxilliped, (3) number of setae of the maxilliped and of the peraeopods.

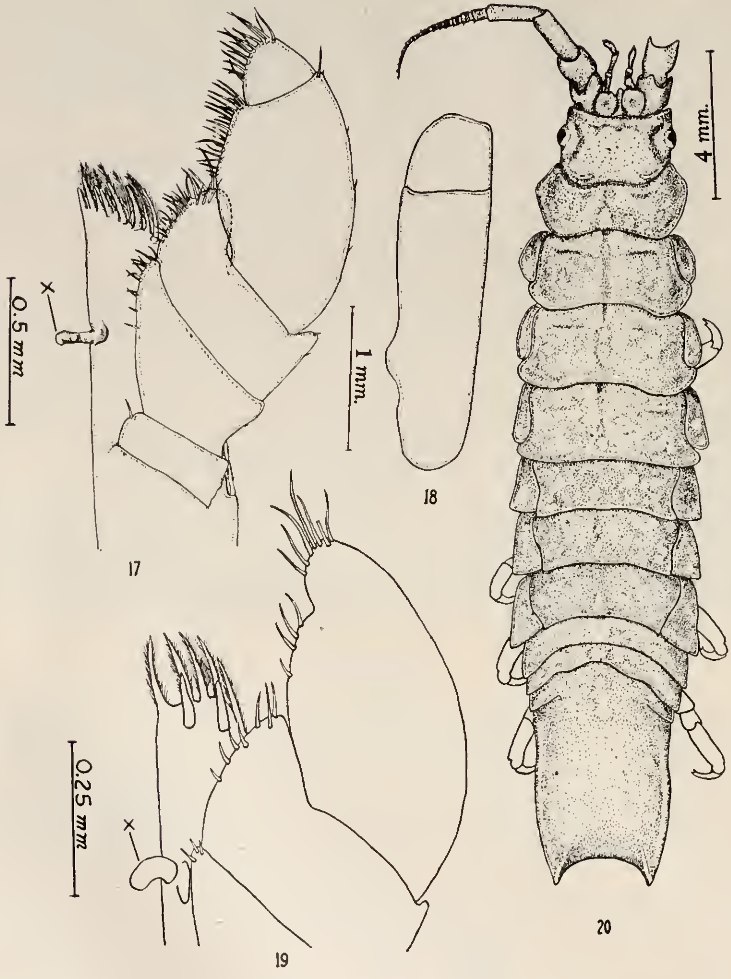
FIGURE 13. Seventh peraeopod, right, juvenile, below 6.0 mm. length. Magnification as per Figure 1.

FIGURE 14. Seventh peraeopod, left, juvenile, above 6.0 mm. length. Magnification as per Figure 1.

FIGURE 15. Propodus and dactylus of seventh peraeopod, small adult male, 9.5 mm. length. Magnification as per Figure 1.

FIGURE 16. Ventral border of propodus of seventh peraeopod of large adult male, 20.5 mm. length. Magnification as per Figure 1.

PLATE III



Pentidotea resecata

Magnification as indicated

FIGURE 17. Maxilliped, left, small adult male, 9.5 mm. length; "x" is the coupling-hook.
FIGURE 18. Uropod, left, small adult male, 9.5 mm. length.
FIGURE 19. Maxilliped, left, juvenile, 5.2 mm. length; "x" is the coupling-hook.
FIGURE 20. Dorsal view, adult male, 20.5 mm. length.

It would seem to be necessary, in view of the findings, to reexamine the status of species and genera which owe their existence exclusively or in part to characteristics here shown, in this species at least, to be variable in different age groups.

One species *Idothea rufescens* Fee, apparently based on immature specimens, is considered a synonym of *Pentidotea resecata* (Stimpson).

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THE UTILIZATION OF SUGARS AND OTHER SUBSTANCES BY DROSOPHILA

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Studies have been made of the use of carbohydrates and other food material by several insects, e.g. the honey-bee (Bertholf, 1927; Phillips, 1927; Vogel, 1931), the blow-fly (Fraenkel, 1936, 1940), the Mexican fruit fly, *Anastrepha ludens* (Baker et al., 1944), and a number of others. The reviews of Trager, 1941 and 1947, and Uvarov, 1928, furnish extensive references. *Drosophila melanogaster* seems, however, to have escaped attention in this connection heretofore. Experiments have now been made on the ability of this fly to utilize a large number of carbohydrates and related compounds, as well as some substances of other classes. In addition, an estimate of the relative nutritional efficiency of these substances has been made.

MATERIAL AND METHODS

Adults. To rear flies for these tests, the standard corn meal, agar, and sugar medium, in half-pint milk bottles, with an inoculation of fresh yeast, was used. As soon as the larvae reached full size and began to leave the medium, a layer of sawdust was added. This prevented the adults from obtaining any food until they were transferred to test bottles. The flies were used as soon as possible, never more than 24 hours after emergence.

Test bottles were set up as follows: solutions to be tested were put into 10 ml. vials stoppered with a roll of filter paper which served as a wick. About 50 ml. of 1.5 per cent agar was poured into a half-pint milk bottle: this maintained moisture and facilitated counting dead flies. For non-fermentable substances the vials were simply embedded in the agar base, otherwise they were wrapped in strips of paper toweling to form a plug for the milk bottle. This stopper could be changed readily and fresh solutions offered the flies, eliminating the complications of bacterial growth. It was found desirable to transfer the flies to fresh bottles after about two weeks if they survived, since otherwise dead flies were eaten by larvae and counting became difficult.

One hundred flies were used for each test. They were divided among three bottles for convenience in counting. The dead flies in the bottles were counted each day. Initially the number of days required for 50 per cent of the flies to die was used as a means of evaluating the degree of utilization of a substance, but it was found that many of the materials having low values could not be differentiated without making counts at shorter intervals, which was impractical. A better index was achieved by totalling the daily survival percentages and using the resulting number as an index of nutritive value. For example, when formic acid was fed to flies, all survived the first day, 43 per cent the second, none the third. The "score" was, therefore, 143.

Larvae. Three of the common sugars were tested on sterile larvae. Eggs were obtained by allowing flies to deposit them on small dishes of agar for about two hours; the eggs were then collected and sterilized by immersion in 85 per cent alcohol for 10 minutes and transferred to shell vials containing 10 ml. of sterile culture medium. Each vial contained the following: powdered agar, 150 mg.; dried brewer's yeast, 50 mg.; sugar, 50 mg.; distilled water, 10 ml. The same medium, minus sugar, is the "starvation diet" of Beadle et al. (1938), and this, together with their "adequate" diet of 2 per cent yeast, was used for comparison with the sugar supplemented media.

Each vial was seeded with 40 eggs and maintained at 25° C. After the formation of pupae, the vials were examined daily and when all the adults had emerged, counts were made to ascertain: (a) number of adults; (b) number of pupae not completing metamorphosis; (c) number of unhatched eggs. The larvae sometimes churned the medium so that unhatched eggs were lost, but a large number of vials were found with eggs and egg cases undisturbed; from these it was calculated that an average of 4 eggs per vial failed to hatch. The numbers of eggs given in Table IV represent, therefore, 36 eggs per vial.

RESULTS

If flies are put into dry bottles, they are all dead within 48 hours: their score is 65. If a layer of agar is put into the bottles, the score is 110; if, in addition, a vial of distilled water is supplied, the score rises to 120. On standard corn meal, agar, and sugar medium, they live a long time: the score for that is 4418.

Table I shows the scores calculated as described above, and the day on which 50 per cent of the flies in each test were left alive. From the data it can be seen that adults of *Drosophila melanogaster* can live on a large number of substances in several classes of chemical compounds, but that the sugars and their close derivatives are best for maintaining these insects. Even in the sugars, each subgroup is found to contain substances which cannot be utilized.

If flies are supplied with pure sugar solutions, they survive for periods dependent upon the degree of utilization of the sugar and its concentration. Poorly utilized sugars like xylose sustain life only for short periods, even in concentrated solutions, while well utilized sugars like sucrose maintain life for longer and longer periods as the concentration increases. The limit in this direction seems to be reached between M/10 and M/5 for sucrose, for further increases in the concentration fail to increase survival. Groups of flies tested with concentrations of sucrose as follows: M/5, M/2, M, and 2M gave results no better than M/10, and indeed, the higher concentrations showed a tendency to decrease the life span slightly, but other factors such as osmotic pressure might enter to account for this.

The substances which were tested gave scores ranging from that of raffinose, 2600, to guanine, 13, as shown in Table I. Three groups of substances can be distinguished:

Group 1. Substances which appear to be inert, with scores close to that of water. Because of the natural variability of different batches of flies, and temperature conditions as noted previously, one could not expect sharply demarcated groups, and in fact there is a continuous gradation of scores. Probably all substances with scores between 100 and 150 should be called inert. This group would

TABLE I

The survival of adult Drosophila melanogaster on various substances, given as summations of daily survival percentages (A), and as days required for 50 per cent mortality (B). Except where noted, solutions are M/10. Each test represents 100 flies.

	A	B
Controls		
Dry bottle	65	1
Bottle with agar	110	2
Water (442 flies)	120	2
Standard medium	4418	45
Pentoses		
D-Xylose, M/2	680	7
Ribose	340	4
D-Xylose	211	3
L-Fucose	169	3
D-Arabinose	166	3
D-Xylose, M/20	131	2
L-Arabinose, M/2	101	2
L-Rhamnose, M	80	2
D-Arabinose, M/2	69	2
L-Rhamnose	68	2
L-Arabinose	64	2
Hexoses		
D-Fructose	1855	18
Glucose	1521	16
D-Mannose	1415	14
D-Fructose, M/20	1033	11
D-Galactose	945	9
Gluctose, M/20	663	7
D-Galactose, M/20	235	3
L-Sorbose	191	3
L-Sorbose, M/2	68	2
Disaccharides		
Sucrose	2218	24
Sucrose, M/5	2141	23
Maltose	2040	17
Sucrose, M	2010	22
Trehalose	1864	21
Maltose, M/20	1668	16
Sucrose, M/2	1624	20
Sucrose, 2M	1516	16
Sucrose, M/20	1506	14
Melibiose	1237	12
Sucrose, M/40	382	4
Lactose	179	3
Lactose, M/2	153	2
Lactose, M/20	100	2
Cellobiose	84	2
Cellobiose, M/2	40	1
Trisaccharides		
Raffinose	2600	28
Melezitose	2432	26
Raffinose, M/20	1460	15
Melezitose, M/20	909	14
Polysaccharides		
Dextrin, 1%	778	8
Starch, 1%	334	4
Glycogen, 1%	298	4
Inulin, sat. sol.	160	2
Alcohols		
Ethyl, M/5	172	3
Ethyl, M/2	99	2
Ethyl, M/10	93	2
n-Butyl	102	2
tert-Amyl	100	2
n-Amyl	99	2
iso-Butyl	96	2
sec-Butyl	95	2
tert-Butyl	50	
Polyhydric alcohols		
Glycerol	1369	14
Mannitol	729	6
Inositol	572	6
Sorbitol	358	5
Adonitol	308	4
m-Erythritol	170	3
Dulcitol, M/5	119	2
Dulcitol	108	2
Arabitol	107	2
m-Erythritol, M/2	86	2
penta-Erythritol,		
M/2	51	1
M/10	40	1
Glycols		
Propylene	172	3
Diethylene	160	2
Ethylene	124	2
Dipropylene	60	2
Carboxylic acids		
Butyric	205	3
Acetic	202	3
Formic	143	2
Valeric	133	2
Propionic	113	2
Lactic, M/2	377	5
M/5	327	4
M/10	208	3
M/20	153	2
Pyruvic, M/5	100	2
M/10	90	2
M/20	75	2
Glycolic	107	2
Levulinic	97	2
Succinic	367	4
Pimelic	160	3
Glutaric	124	2
Malonic	88	2
Azelaic	80	2
Adipic	70	2
Oxalic	20	1
Malic	234	3
Aconitic	162	3
Itaconic	158	3
Fumaric	151	2
Maleic	120	2
m-Tartaric	97	2
Citric	413	4
Salts		
Sodium succinate	115	2
Sodium citrate	105	2
Sodium lactate	115	2
Sodium malonate	99	2

TABLE I—*Continued*

	A	B		A	B
Amino acids			Miscellaneous		
Glycine	202	3	Yeast-sucrose, equal parts, dry	2074	24
DL-Methionine	195	3	alpha-Methylglucoside	639	6
L-Glutamic acid	124	2	Yeast, fresh 2% suspension	165	3
DL-Aspartic acid	122	2	Parenamine, 1% (proprietary casein hydrolysate)	147	2
DL-Alanine	108	2	Amygdalin	139	2
Beta alanine	108	2	Yeast, fresh dry	128	2
L-Cystine (sat. sol.)	102	2	Catechol	126	2
L-Cysteine	101	2	Albumin, 1%	117	2
DL-Glutamic acid	101	2	Lecithin, 1%	116	2
DL-Threonine	101	2	Charcoal, dry	107	2
L-Arginine	95	2	Glucosamine	106	2
DL-Phenylalanine	93	2	Casein, dry	106	2
L-Histidine	89	2	Gulonic lactone, 4%	105	2
DL-Isoleucine	72	2	Magnesium hexosediphosphate	104	2
L-Lysine	71	2	Glucoheptonic lactone, 4%	100	2
L-Proline	70	2	D-Galacturonic acid	98	2
L-Leucine (sat. sol.)	67	2	Xylan (sat. sol.)	94	2
L-Hydroxyproline	66	2	Sucrose acetate	93	2
DL-Tryptophane (sat. sol.)	63	2	Mucic acid	90	2
L-Tryptophane (sat. sol.)	62	2	Calcium glucoheptonate, 4%	84	2
L-Tryosine (sat. sol.)	57	2	Nucleic acid (sat. sol.)	83	2
DL-Leucine (sat. sol.)	55	2	Sodium nucleate, 1%	82	2
DL-Norleucine	55	2	Yeast, dried, suspension	80	2
DL-Serine	51	1	Milk, powdered	78	2
DL-Valine	51	1	Yeast, dried	64	2
			Starch, Lintner, dry	45	1
			Xanthine (sat. sol.)	15	1
			Guanine	13	1
			Uracil	13	1

include not only substances not utilized when ingested, but those which might be utilized somewhat, were they not also slightly repellent so that the flies do not drink the solutions.

Group 2. Substances which are utilized by Drosophila, shown by scores higher than that of water. This group includes anything which prolonged the life of the flies in any degree, from such poor nutrients as xylose to the best of the higher sugars. Sugars, particularly the mono-, di-, and trisaccharides, lead in this group, but moderately good results were obtained with dextrin, glycerol, mannitol, inositol, and alpha-methylglucoside. Some prolongation of life was obtained with starch, glycogen, sorbitol, adonitol, and with butyric, acetic, lactic, succinic, malic, and citric acids. The only amino acids showing any usefulness were methionine and glycine. A few other substances, such as ethyl alcohol, propylene and diethylene glycol, aconitic and itaconic acids, were doubtful. Proteins alone, e.g. albumin, were of no value, nor were such products as casein, yeast, or milk. The low values obtained with dry yeast (64) and starch (45) prompted a test with an inert powder. Charcoal was selected, and the relatively high score (107) suggests that there is something definitely harmful in dry starch and yeast, but whether its nature is physical or chemical has not yet been ascertained. Dry yeast mixed with an equal amount of powdered sugar, on the other hand, makes an excellent food, giving a score of 2074.

In order to obtain a more exact comparison of nutritive value among some of the commoner sugars, seven were tested under identical conditions. The molarities of the solutions were chosen to equate the mono- and disaccharides with respect to weight per unit volume. Lactose, M/20, and xylose, M/10, showed no nutritive value, and galactose, M/10, very little. The other sugars were, in order of increasing nutritive value: glucose, M/10, 1375; sucrose, M/20, 1440; maltose, M/20, 1720; fructose, M/10, 1833. These scores and the curves of Figure 1 show there was little variation in this group, also that the results were nearly the same as those shown in Table I for the larger series of experiments.

The longevity of flies fed on di- and trisaccharides was compared, under identical conditions, with that of flies fed on the constituent monosaccharides. Table II shows that there was little difference in the results, a mixture of fructose and glucose being as good as an equivalent amount of sucrose, etc.

TABLE II

A comparison of some di- and trisaccharides with their hexose constituents. Each pair was run with flies from the same batch, under identical temperature conditions.

Substance	Conc.	Score	Substance	Conc.	Score
Sucrose	M/20	1455	Raffinose	M/20	1460
Fructose Glucose	M/20 M/20	1421	Fructose Glucose Galactose	M/20 M/20 M/20	1492
Maltose	M/20	1466			
Glucose	M/10	1363	Melezitose	M/20	1257
Trehalose	M/20	1064	Glucose Fructose	M/10 M/20	1285
Glucose	M/10	1285			

Larvae. The results obtained in rearing sterile larvae on yeast and on yeast-sugar mixtures are given in Table IV. No significant difference was found in the number of flies produced by the three sugar media. A significant difference was found when adequate amounts of yeast were supplied, and an increase in the amount of sugar might have increased the yield. Since the object of the experiment was to differentiate among the sugars, if possible, by putting the larvae into somewhat unfavorable conditions, this was not done. Flies consuming fructose developed more rapidly than those on sucrose and glucose, though less rapidly than those having a full yeast diet.

Group 3. Substances which have low scores, and are therefore toxic or repellent. Flies in a bottle having a layer of agar live almost as long as if they are supplied with drinking water. Substances which are merely repellent will, therefore, be difficult to separate from those which are nutritionally inert. Toxic substances should give much lower scores and be accordingly easier to single out. Guanine, for example, is clearly toxic. Variations in toxicity and in the flies themselves naturally militate against any sharp distinction, so that further experiments were performed to bring out hidden differences. The difference between toxic and repellent substances can sometimes be demonstrated readily by offering a questionable solution alone and in combination with a separate vial of water. Rhamnose alone, for example, gave a score of 68, but when the flies were offered an additional vial of water, the score rose to 100. No discrimination was evidenced, and presumably the flies lived longer because they drank less of the rhamnose solution. When repellency is suspected, however, something must be used to insure the ingestion of the solution. Vogel (1931) used sucrose solution, and a M/40 solution of sucrose was found useful in these experiments. Testing a large number of flies with this solution alone gave a score of 382. Table III shows how the results differed when various substances were added to it. Dulcitol alone is seemingly inert in M/10 solution, but when M/40 sucrose is added, the flies live longer than in sugar alone (score 508). Isoleucine is inert either way. D-Arabinose, on the other hand, prolongs life slightly when alone but shortens it when added to the sucrose solution, a puzzling result, to be sure. Sorbose would seem to be toxic either alone or in sucrose solutions, as do tartaric acid, norleucine and histidine, while valine, which is toxic when alone, can probably be detoxified when sucrose is present.

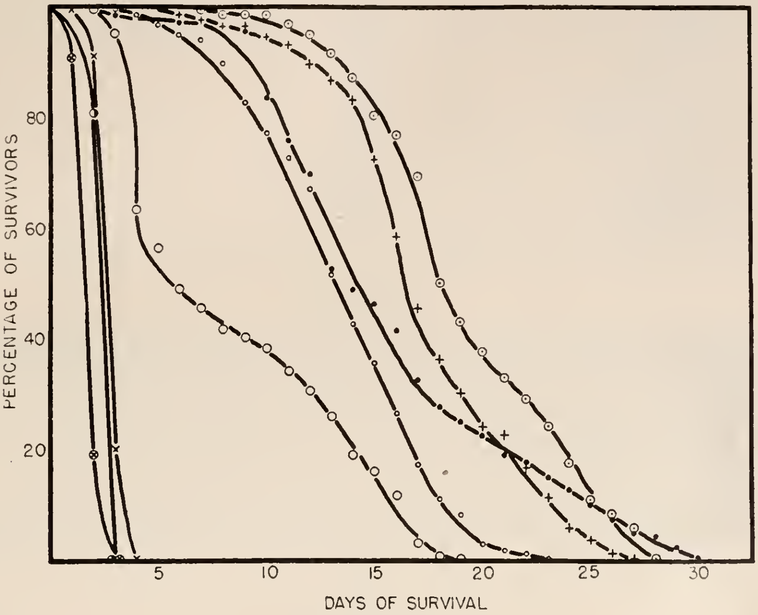

FIGURE 1. The duration of life of adult fruit flies fed solutions of various sugars. Lactose, M/20, ⊗; water, ◑; xylose, M/10, ×; galactose, M/10, ○; glucose, M/10, ○; sucrose, M/20, ●; maltose, M/20, +; fructose, M/10, ⊙.

TABLE III

The effect of certain substances on Drosophila when dissolved in water and in M/40 sucrose. Each pair run under identical conditions.

Substance	Conc.	Score	
		In water	In M/40 sucrose
Cellobiose	M/10	84	396
Dulcitol	M/5	119	508
D-Arabinose	M/5	170	162
L-Sorbose	M/2	68	285
M-Tartaric acid	M/5	102	124
D-Tartaric acid	M/5	80	115
DL-Norleucine	M/10	20	83
DL-Valine	M/10	24	353
DL-Isoleucine	M/10	111	360
L-Histidine	M/10	93	203

DISCUSSION

As noted above, the question of what sugars can be utilized by insects has been investigated for several species. The results in hand for the adult and larval bee, the adult blowfly, and for the adult fruit flies *Anastrepha* and *Drosophila,* indicate almost identical abilities to utilize sugars, as nearly as the data are comparable. The really clear cut differences reported are as follows: mannose is used by *Calliphora, Anastrepha* and *Drosophila,* but not by the bee. Indeed von Frisch (1934) and Staudenmayer (1939) have reported a specific toxicity of mannose for the bee. Melibiose, dextrin, starch, and glycerol are not used by adult bees, but

TABLE IV

The development of sterile Drosophila larvae on low yeast, low yeast plus sugars, and adequate yeast diets.

Medium	Number of eggs (36/vial)	Number of pupae	Mean number of pupae per vial	Difference divided by prob. error of difference	Number of adults	Mean number of adults per vial	Difference divided by prob. error of difference	Mean number of days for emergence of all flies
0.5% yeast	252	73	10.3±2.9*	2.0	67	9.6±3.3*	2.0	20.5±3.4*
0.5% yeast 0.5% glucose	180	106	21.2±4.1	0.8	102	20.4±4.3	0.6	21.0±1.7
0.5% yeast 0.5% sucrose	540	371	28.5±2.3	2.0	353	27.1±2.3	1.4	21.5±1.3
0.5% yeast 0.5% fructose	216	183	30.5±1.0	3.0	178	29.7±1.8	4.5	16.2±3.7
2.0% yeast	72	70	35.0±0.9		70	35.0±0.9		12.0±0.0

* Probable error.

are by *Calliphora* and *Drosophila*. Inositol is utilized by *Drosophila*, but not by the others, and arabinose is used by *Apis* alone. There are other differences reported, such as the use of fucose by *Drosophila* and not by other forms, but the degree of utilization is so small that the difference is unimportant. The present experiments do show, however, that no substance should be judged inert until it has been tested in several concentrations, e.g. xylose is very poor in M/10 or less, but definitely useful in M/2. Also, substances should not be finally classified as useless or toxic unless they are offered in such form that ingestion is certain. Dulcitol, for example, is apparently inert for *Drosophila* when given alone, even up to M/5, yet when it is dissolved in M/40 sucrose, the flies live longer. The comments of Vogel (1931), Haslinger (1935) and Fraenkel (1940) are also pertinent to this point.

The ability of *Calliphora* and *Drosophila* to utilize glycogen and starch is clear, although it is much less than the ability to utilize sugars. The danger of using a partially hydrolyzed starch should be noted. *Drosophila* fed Lintner's soluble starch, one per cent, gave a score of 625, whereas sugar-free corn starch scored only 334. Reducing sugar was readily demonstrated in the soluble starch, which may account for the partial development of *Aedes* larvae reported by Hinman (1933).

The question of which sugar is best, which was raised by Bertholf (1927), is, perhaps, one applicable only to the individual species. It is further complicated by the variety of standards adopted by various investigators. Yet it is interesting to note that the "physiological sugar," glucose, is consistently poorer than others, being rated second by Phillips, third by Baker and Fraenkel, and fourth by Bertholf and in the present experiments, when only sucrose, maltose, glucose, and fructose are considered. Fructose, on the other hand, is rated first by Phillips, equal to sucrose by Fraenkel, second to sucrose by Bertholf, and in the present experiments it was superior to the others. Indeed, a comparison of scores for M/10 fructose and M/20 raffinose indicates that fructose is superior to the trisaccharides also. Sucrose is at or near the top in all.

The curve for galactose in Figure 1 is also of some interest. The initial mortality was so heavy that it suggested reduced powers for utilization of galactose, or greater power of mobilizing enzymes, on the part of one of the two portions of the population. A repetition of the experiment yielded similar results. The basis of the variability is not known but it will be investigated.

Partial successes were obtained with the substances regarded as intermediate products of carbohydrate metabolism. None of these was utilized by *Calliphora* (Fraenkel); *Drosophila*, however, survives a short time on citric, malic, succinic, lactic, butyric, and acetic acids, and possibly also on aconitic, itaconic, fumaric, and pimelic acids, although these are on the borderline. Since there is such close agreement in other respects, these data suggest that the blowfly might be able to metabolize the compounds in question, a possibility which Fraenkel has pointed out. In an experiment in which the present technique was used with *Lucilia sericata*, the flies died about as rapidly when offered M/10 citric acid or dry citric acid as they did when offered water alone. *Calliphora* was not available for this test, but the results with *Lucilia* suggest that if blowflies are able to metabolize any of the intermediates, some other means must be employed for introduction of the material.

According to Weidenhagen (1931), and the somewhat modified point of view of Pigman (1944), all carbohydrates can be split by a small number of enzymes. With Weidenhagen's work in mind, Fraenkel concludes that only two enzymes, an alpha-glucosidase and an alpha-galactosidase, need exist in *Calliphora* to split all the carbohydrates that the blowfly utilizes. *Drosophila* evidently depends largely on the same two, but may have in addition a fructofuranosidase, which would be needed to utilize inulin, and could also act on sucrose. An amylase, too, must be present to split starch and glycogen.

While the longevity of the fruit fly on sugar alone may seem remarkable (50 per cent survival up to four weeks), the much greater longevity on the standard culture medium which furnishes carbohydrate directly and protein and accessory factors from the yeasts growing on the medium suggests that the addition of traces of other substances to the sugar solution might increase survival greatly. A further point on longevity is that the present method is not calculated to produce the longest lived flies. According to Pearl, Miner and Parker (1927), the maximum longevity of *Drosophila* is found in relatively crowded populations, about 50 flies in a 30 ml. vial having given best results in their experiments.

SUMMARY

1. *Drosophila melanogaster* can survive for varying periods on pure solutions of many compounds, including sugars, polysaccharides, polyhydric alcohols, aliphatic acids, etc.

2. In equivalent solutions, the order of usefulness of some common sugars was found to be: fructose > maltose > sucrose > glucose $\geq$ galactose > xylose > lactose.

3. There is no significant difference in life span between flies fed on disaccharides and their constituent monosaccharides.

4. Doubtful sugars can usually be resolved into toxic, repellent, or slightly useful substances by offering them in dilute sucrose solutions.

5. On a sterile, "starvation" diet, larvae develop better on fructose than on glucose.

6. On the basis of survival when fed pure substances, *Drosophila* seems to possess alpha-glucosidase, alpha-galactosidase, beta-fructofuranosidase and amylase.

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RESPIRATION OF OOCYTES, UNFERTILIZED EGGS AND FERTILIZED EGGS FROM PSAMMECHINUS AND ASTERIAS

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1. INTRODUCTION

Using Cartesian diver micro-respiration technique Lindahl and Holter (1941) measured the oxygen consumption rate of primary oocytes, mature unfertilized eggs and fertilized eggs of the sea-urchin *Paracentrotus lividus*. They found that the oocyte respiration is markedly higher than that of the unfertilized egg and that it probably exceeds that of the newly fertilized egg. On the other hand Boell, Chambers, Glancy and Stern (1940) stated, in an earlier brief note, that in similar diver measurements the mature unfertilized *Arbacia* egg reveals a higher oxygen consumption and a higher capacity to oxidize p-phenylenediamine than does the immature egg (oocyte).

Because of these two quite divergent results it was considered to be of interest to investigate, and eventually settle the matter, by using a third sea-urchin species. *Psammechinus miliaris* from the Swedish West Coast was chosen. This species offers some special advantages: its spawning period is relatively long; its oocytes can be obtained regularly during practically the whole of the spawning period; and, there are three distinct cytoplasmic maturity stages of the egg, viz., under-ripeness, ripeness and over-ripeness. These maturity stages are characterized by differences in fertilizability, in fertilization membrane appearance and in reaction to hypertonic medium (cf. Runnström and Monné, 1945). Thus it might be possible to follow in detail any gradual alteration in oxygen consumption during the entire egg maturation process. Since it is necessary selectively to pick out the desired cells from the cell mixture, which is extruded from the ovary, the diver technique, which permits the measurement of the oxygen consumption of as few as about a hundred oocytes or resting eggs, will be very suitable. As only a small number of cells are necessary for each experiment, it is possible to charge any desired parallel diver unit with cells from the same ovary.

Furthermore, it was thought that a comparison between the sea-urchin oocyte or egg respiration and that of the starfish oocyte or egg might throw some light on the general laws of echinoderm egg metabolism. Tang (1931), using a Warburg technique, found that mature and immature (oocytes) *Asterias* eggs used up the same oxygen amount per time unit. Boell, Chambers, Glancy and Stern (1940) arrived at the same result using diver technique, but Brooks (1943) in Warburg experiments found a lower consumption in immature eggs than in mature. In the present paper diver measurements on *Asterias glacialis* oocytes and eggs will be reported.

In addition, diver measurement data concerning the oxygen consumption of the fertilized sea-urchin and starfish egg are given and interpreted. In *Asterias* the respiration is only followed over the first mitosis, but in *Psammechinus* it is measured until some hours after hatching.

Abbreviations used in the text:
Ps.: *Psammechinus miliaris*
Ast.: *Asterias glacialis*
Par.: *Paracentrotus lividus*

2. GENERAL REMARKS ON MATERIAL AND METHODS

2.1. *Animals*

Ps. occurs in two phenotypic varieties: one, called the *Z-form,* is the trivial littoral form; the other, called the *S-form,* is found at greater depths. They differ in size and morphological appearance and, of special interest in this investigation, in the spawning period. The *Z-form* has fertilizable eggs from the middle of June until the middle of July, the *S-form* during July and August. Concerning living conditions, distribution and biology, cf. Lindahl and Runnström (1929) and Borei and Wernstedt (1935).

The *Ps. Z-form* animals were dredged from about 6 m. depth. They were kept in a wire mesh cage immersed in the surface water off the station pier, where temperature and salinity conditions were approximately the same as those at the dredging-locality. The sea-urchins were used for experiments within a few days of being caught.

The *Ps. S-form* sea-urchins were caught at about 20–30 m. depth. They were brought to the station immersed in 32–33 ‰ salinity water in a big Dewar vessel to keep the temperature low. At the station they were transferred to aquaria with running sea water, where the salinity was about 32–33 ‰ and the temperature between 15–17° C. The conditions at the dredging-localities were about the same with regard to salinity, but somewhat lower as regards temperature. The animals were mostly used for experiments on the day of capture; in some rare cases they were not used until the next day.

Ast. was dredged from 30–40 m. depth. The animals were brought to the station and kept there in the manner described above for the *Ps. S-form.* They were invariably used for experiments on the day of capture. Their spawning period falls mainly in May and June.

2.2. *Diver technique*

The technique of Cartesian diver measurements has been described in detail by Holter (1943). Only points of special interest will be mentioned here.

The divers used were of standard type (volume 8–10 μl.) made of Jena Geräte glass ($\phi = 2.412$). They were charged as follows, according to the "Diver charge Type I" of Borei (1948):

Mouth seal: Holter's medium ($\phi_M = 1.325$)
Neck seal I: 0.5 μl. paraffin oil ($\phi_{oi} = 0.87$)

$$\left.\begin{array}{l}\text{Neck seal II: 0.5 }\mu\text{l. isotonic sodium hydroxide}\\ \text{Bottom drop: 0.8 }\mu\text{l. sea water cell suspension}\end{array}\right\}(\phi_W \preceq 1.0)$$

(cf. Borei (1948), Figure 1: I).

The cells were extruded from the ovary after this had been removed from the body, sifted through bolting-cloth and washed three times in sea water before being picked up in a braking pipette.

The salinity of the sea water of the cell suspension varied according to the material. For the *Ps. Z-form* water of 24.6 $^0/_{00}$ S was mostly used. This salinity figure approximately equals the medium salinity during the summer months of the surface water off the station pier where the animals were kept. Occasionally higher salinities occur. Thus it was sometimes found more correct to apply water of 27 or 29 $^0/_{00}$ S. For the *Ps. S-form* which lives in water of higher salinity and was therefore kept in the station aquarium sea water; 32–33 $^0/_{00}$ S sea water was used. In the *Ast.* experiments the salinity was 29 $^0/_{00}$ throughout. This salinity is somewhat lower than that on the dredging-localities, but had to be used owing to some temporary trouble with the station sea water pipe-line. All salinities were checked by titrimetric estimations according to Borei (1947). For pH control potentiometric measurements (glass electrode) were employed. Isotonic NaOH solutions for the diver neck seal II were prepared from a stock solution. (From the sea water freezing points tabulated by Knudsen (1903) it can be calculated that 0.365 N NaOH is isotonic to 25 $^0/_{00}$ S sea water.)

The temperature in the experiments was mainly 18° C. For some *Ps.* experiments temperatures between 15–21° C. were employed, owing to the requirements of simultaneous measurements for other investigations. The maximum temperature for normal larval development of *Ps.* was studied by Runnström (1927) and found to be 22° C. *Ast.* belongs to the same species group, the mediterranean-boreal, and is likely to have about the same upper temperature limit for normal development. The temperatures in the experiments are thus well below the critical level.

The number of eggs per diver was 40–50 for *Ast.*, 70–120 for *Ps.* when unfertilized, and 40–50 for *Ps.* when fertilized. These numbers give approximately 12.5, 8 and 9×10^{-3} μl. oxygen consumed per hour respectively in the most crowded divers, *i.e.* δp lies between 1–2 cm. per hour. This rate is best suited to keep the errors of the diver apparatus low (cf. Holter, 1943) and lies, moreover, within the range $3\text{–}18 \times 10^{-3}$ μl. per hour, which Lindahl and Holter (1940) in diver experiments on *Par.* found to be characterized by direct proportionality between number of cells and oxygen consumption. Lindahl and Holter (1940) further found that diver and Warburg experiments which were performed simultaneously gave very consistent results. The same applies to the present diver experiments on unfertilized *Ps.* eggs compared with the Warburg experiments of Borei (1934) (cf. 3.112.2). Thus the oxygen supply is apparently not the limiting factor in these diver experiments. This view is further supported by the fact that in the course of the experiments the oxygen pressure within the diver does not decrease more than 2 mm. Hg at the most, whereas it is generally agreed (cf. Tang, 1941) that the sea-urchin egg respiration is unaffected by a decrease in oxygen pressure from 160 mm. down to 40 mm. The number of cells per volume of cell suspension is about the same in diver experiments as in Warburg ones, or slightly lower.

After completed diver measurements the cells were washed out of the divers with sea water, re-counted and then microscopically observed as to condition and development. In applicable cases even fertilizability controls were undertaken. Only those experiments were accepted in which the cells passed these post-diver-measurement controls.

2.3. *Evaluation of results*

The oxygen used up during the experiment, δv, is calculated from the read pressure difference, δp, according to the formula

$$\delta v = \frac{V \cdot \delta p \cdot T_0}{p_0 \cdot T}. \tag{1}$$

Using this formula δv will be given in μl. measured at 0° C. and normal barometric pressure, provided δp is stated in cm. Brodie, p_0 is the normal barometric pressure in cm. Brodie (= 1000), T is the temperature of the experiment in °K., T_0 stands for 273° K., and if V stands for the total gas space in μl. of the charged diver at equilibrium pressure. It will be noted that this formula is similar to that used by Holter (1943) p. 466 in cases where the solubility of the measured gas is low, and where formed CO_2 is absorbed away, but a temperature correction has been added in order to render a comparison between the measurements possible even if the latter have been taken at different temperatures (see, however, below). In the formula the absorption of oxygen in the liquid phases of the charge has been disregarded as it is a very small quantity.

From the diver equation given by Linderström-Lang (1943) p. 363 the following expression for V may be derived:

$$V = g_D\left[\frac{1}{\phi_M} - \frac{1}{\phi_{Gl}}\right] + V_W\left[\frac{\phi_W}{\phi_M} - 1\right] + V_{Oi}\left[\frac{\phi_{Oi}}{\phi_M} - 1\right] \qquad (2)$$

where g_D = weight of the empty diver in mg.,
V_W = volume in μl. of aqueous charge (*i.e.* in the present case: cell suspension in bottom drop + hydroxide solution in neck seal II),
V_{Oi} = volume in μl. of paraffin oil in neck seal I,
ϕ_W, ϕ_{Oi}, ϕ_M and ϕ_{Gl} = densities of the aqueous charge, the paraffin oil, the medium and the diver glass.

The formula may be shortened to:

$$V = g_D \cdot A + B + C \qquad (3)$$

where $g_D \cdot A$ may be defined as the total gas space in μl. of the uncharged diver at equilibrium pressure. This is a constant characteristic of the individual diver. For calculating A the graph in Figure 1a may be of help. For a given medium (in this investigation Holter's medium, $\phi = 1.325$; cf. Holter (1943) p. 412, has been used) A is solely a function of ϕ_{Gl}.

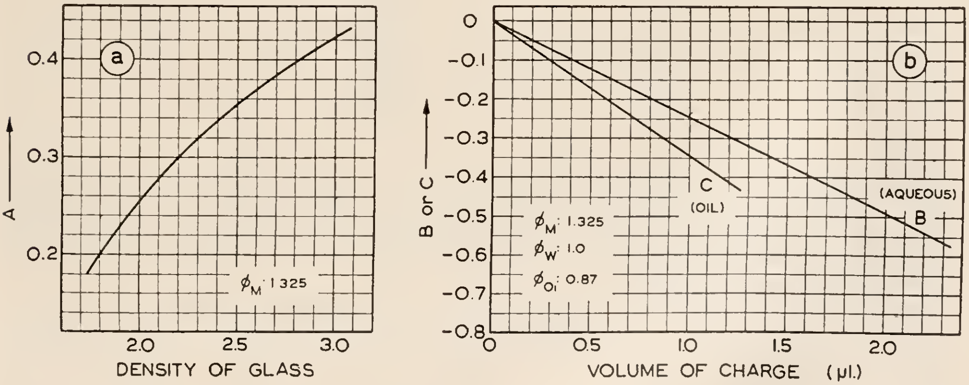

FIGURE 1. Graphs for calculating the values of the constants A, B and C in the formula for V, the total gas space of the charged diver at equilibrium pressure.

The constants B and C are governed by the adopted type of charge, but are independent of the characteristics of the diver. They may be found from the graphs in Figure 1b.

The value of A ought to be stated to the third figure; B and C will be sufficiently correct if stated to the second.

As stated in 2.2., measurements were usually performed at 18° C., but in some experiments on *Ps.* cells the temperature was varied within the limits 15–21° C. Measurements at different temperatures are not comparable if merely calculated according to formula (1), as the cellular oxygen consumption rate is itself a function of temperature. As will be shown in a later paper (jointly with S. Lybing) this function in the unfertilized *Ps.* egg is characterized by a $Q_{10} \backsimeq 2.25$. Adopting this value, the consumption rates at any temperature within the range may be converted to consumption rates at 18° C. The same applies when the results of other investigators are compared with those given in this paper. The same Q_{10} value may, without significant errors, be used even for fertilized eggs.

If all corrections and constants are now put together the consumption formula (1) is simplified to:

$$\delta v = V \cdot \delta p \cdot K. \tag{4}$$

The value of K for different experimental temperatures when adopting $Q_{10} = 2.25$ will be found from Table I.

The technical errors of the method will no doubt remain below 5 per cent (cf. Holter, 1943). The biological scattering of the gained figures is, however, much greater. (This scattering is frequently met with by investigators who study oxygen consumption in marine invertebrates. Thus, using apparently identical objects one frequently comes across biological scattering amounting to the horrifying value of over 100 per cent.) In order to get a measure on the biological scattering in this investigation the standard deviation, σ, has been used. This figure gives the limits within which about two-thirds of the experimental results will fall. In σ, of course, the technical errors of the method are incorporated. In addition, the significance of the given average values is stated by the standard error of the mean, ϵ, which gives the limits within which the real center of the biological (and technical) scattering is situated with a probability of about 70 per cent.

TABLE I

Value of K in the ultimate diver gas exchange formula if $Q_{10} = 2.25$

Temperature in experiment ° C.	K
14.5	1.264×10^{-3}
15	1.210
16	1.111
17	1.020
18	*0.938*
19	0.862
20	0.792
21	0.728
22	0.670
23	0.618

For *Par.* Lindahl and Holter (1940) state that there is a biological scattering in the egg volume of 10–15 per cent, in the enzyme content of the egg of about 10 per cent, and in the oxygen consumption (obtained by diver technique) of 10 per cent. In the respiration of *Ciona* eggs Holter and Zeuthen (1944) in diver experiments found a scattering of less than 20 per cent, and in the egg volume less than 16 per cent. (Cf. Zeuthen (1947a), who on pp. 44–48 has discussed the influence of biological factors on metabolic measurements, especially on such as are performed with diver technique.)

In the present investigation the standard deviations for respiration measurements are found in Tables II, III, V and VIII, and for egg size in Tables IV and VI. To summarize it may be stated that for *Ps.* respiration there has been found a relative σ slightly greater than 20 per cent, and for *Ast.* a σ slightly greater than 15 per cent. The scattering in size is markedly smaller: for cell diameter $\sigma = 4.3$ per cent in *Ps.* and 3.5 per cent in *Ast.*, and for cell volume 13.5 per cent and 10.9 per cent, respectively.

3. Experiments and Interpretations

3.1. *Respiration of oocytes and unfertilized eggs*

3.11. *Psammechinus*

3.111. *Cell material*

In *Ps.* the egg may still be unfertilizable though full nuclear maturity has been reached, *i.e.* the polar bodies are expelled. This unfertilizability is due to the well known fact that in sea-urchin eggs nuclear maturity is not followed by cytoplasmic maturity until considerably later. Such cytoplasmically immature *Ps.* eggs are characterized in the hypertonicity test (cf. Runnström and Monné, 1945) by rapidly appearing wrinkles which smooth out very slowly. These eggs are termed in this paper *under-ripe eggs.* When both nucleic and cytoplasmic maturity is reached, and the eggs can accordingly be fertilized, the wrinkles appear in 2–6 minutes after the eggs have been placed in hypertonic medium (2 ml. sea water + 0.6 ml. 2.5 *N* NaCl) and smooth out in about 40 minutes. Such totally mature eggs are here termed *ripe eggs.* Later on the eggs again cease to be fertilizable. The wrinkles of the hypertonicity test are now poor or fail to appear and, if any do appear, they smooth out rapidly. These eggs are termed *over-ripe* (cf. also Wicklund, 1947).

These three classes of egg maturity are not mixed in one and the same female but in a catch of animals, females belonging to all these classes may simultaneously be found. In the beginning of the spawning season the females with under-ripe eggs prevail. At the end of the season not only females with over-ripe eggs become frequent, but there is also a marked rise in the number of females with under-ripe eggs (tendency to second spawning period?). This corresponds to the fact that the number of oocytes in the extruded egg-mass is higher both at the beginning and at the end of the spawning season than at its height.

The size of the oocytes varies greatly. Only such as were fully grown were accepted for diver measurements. To facilitate this such egg material was preferred as showed low oocyte percentage, *i.e.* where the oocytes were already mainly transformed into eggs. Such a state seems to ensure full growth in most of the remaining oocytes. During the diver measurements some of the oocytes occasionally started meioses which, when the eggs were examined after the experiment, could be judged from the broken down nuclear membrane. Such circumstances, however, could never be proved to effect perceptibly the oxygen consumption figures. The number, if any, of developing oocytes was always low (cf. also Lindahl and Holter, 1941).

From the *Ps.* material it was accordingly possible to pick out four different kinds of cells for diver experiments, viz. oocytes, under-ripe eggs, ripe eggs and over-ripe eggs.

In some few cases cells deprived of their jelly hull (treatment with acid) were used because they acted as controls in other experiments on naked cells. According to Borei (1948) there is, however, very little difference, if any, in oxygen consumption between coated and naked cells.

3.112. *Respiration of ripe eggs*

3.112.1. *Respiration curve*

In the ovary the germinal cells are continuously supplied with fresh metabolic material and may thus keep their respiratory rate at a constant level. When shed they are cut off from any additional supply and their metabolic rate is bound to drop gradually. This view is supported by the experimental results; see Figure 2.

The earliest respiration values after removal from the ovary cannot be stated with the diver technique. The time required for washing and picking out the cells, for filling the diver, plus a minimum of time for attaining temperature equilibrium and then for obtaining two measurements suitably apart, cannot be pressed down to much under 40 minutes. The earlier values may, however, be found by extrapolation, but the most interesting period, viz. the shedding, will only be described rather approximately by this method.

A gradual decrease in the oxygen consumption of the shed unfertilized egg has already been reported for the *Ciona* egg by Holter and Zeuthen (1944) using Cartesian diver technique. Lindahl and Holter (1941) in their diver experiments on *Par.* have not stated the time after removal from the ovary, but point out that it would probably have been of importance. In fact, the results presented in Figure 2 show how necessary such a precaution is if comparable values are desired.

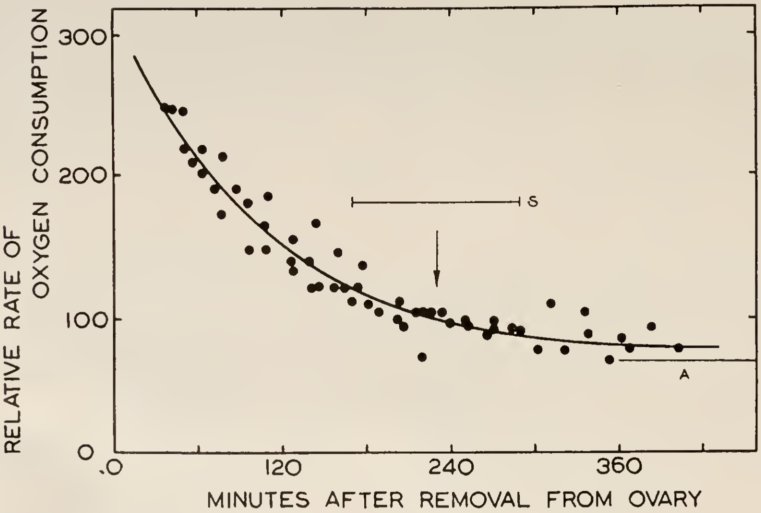

FIGURE 2. Relative rate of oxygen consumption of ripe *Psammechinus miliaris* eggs in relation to time after removal from ovary.

Results from 4 diver experiments. Values at 230 minutes (cf. 3.112.2) put = 100. Final asymptote (A) indicated. Arrow stands for chosen point of comparison for absolute rate measurements. Evaluated time span (S) in such experiments indicated.

The curve in Figure 2 closely resembles that of the endogeneous respiration of baker's yeast (Borei, 1942) where one is also concerned with cells cut off from external supply of metabolic material; the gradually decreasing respiration is composed of two parts. One proceeds monomolecularly and is thought to be governed by the rapidly decreasing amount of an initially present substrate. The other continues with a constant rate for a considerable time; its rate is thought to be limited by the available amount of an enzyme.

Mathematical analysis of the curve in Figure 2 shows that even the respiration of the unfertilized egg can be described in this way: There is a true monomolecular part of the respiration that rapidly tends to become zero, *i.e.* the curve approaches an asymptote (indicated in the figure). The amount below the asymptote represents the constant respiration part. This may probably be interpreted, as in the case of yeast, as a simultaneous degradation of two different sources of metabolites over partly different enzyme systems.

3.112.2. *Absolute respiration values*

Because of the rapidly decreasing rate of oxygen consumption, as manifested in Figure 2, it is not convenient to compare values which are obtained from different materials during the first hours after the egg-removal from the ovary. Even small time differences may here cause considerable discrepancy in the consumption figure. Later on the curve becomes flatter and suitable for comparison with other experiments. The best thing to do would be to take the asymptote value, but this is impracticable by reason of experiment duration. Instead some point must be chosen on the part of the curve that has become reasonably horizontal, but which is still as close as possible to the point of egg-removal. Judging from the obtained curve, a point of time 4 hours from this event was chosen. In actual experiments 230 minutes from egg-removal was the average time, around which the measurements grouped themselves. Experimental conditions, however, have caused the center of the measurements in certain experiments to shift some 20 minutes in either direction. A total measurement-time of 2 hours, during which some six equilibrium readings could be obtained, was thought fit for the evaluation of the consumption figure. The average position on the curve of the final oxygen consumption figure and the time span of the measurements used for its evaluation are marked in Figure 2.

TABLE II

Average rate (r) *of oxygen consumption of ripe eggs of the two Psammechinus phenotypes 230 minutes after removal from ovary*

n = number of diver experiments, each of which was performed on cell material from different females.

Phenotype	r (µl./egg/hour)	σ	ϵ	n	Difference in r (µl./egg/hour)
Z-form	0.47×10^{-4}	0.12	0.02	25	0.06×10^{-4}
S-form	0.53×10^{-4}	0.17	0.03	39	($\epsilon_{\mathrm{diff.}} = 0.03$)

The results from measurements in this manner on ripe eggs of both the *Ps.* phenotypes are recorded in Table II.

The figures for the two phenotypes differ slightly. The difference is, however, not greater than twice its standard error. Thus it has not been possible to establish any significant difference in oxygen consumption rate between the ripe eggs of the two forms (concerning egg volume, cf. 3.114).

Borei (1934) studied the oxygen consumption of ripe *Ps.* eggs by means of Warburg technique (20° C.) and found, in accordance with the above results, the figure 0.52×10^{-4} µl. per egg and hour (converted to be comparable to the figures in Table II, whereby the egg volume value from Table IV has been used).

Using Barcroft differential manometers (14.5° C.) Shearer (1922b) got a considerably lower value for the ripe egg, viz. 0.20×10^{-4} µl./egg and hour (converted to be comparable to the figures in the present investigation). This may be due to imperfect measuring technique (too tightly packed eggs).

3.113. *Respiration of oocytes, under-ripe eggs and over-ripe eggs*

To get oxygen consumption values comparable to those of the ripe eggs precautions were taken, similar to those mentioned above, concerning the measurements. It was found that even the oocytes showed a decreasing respiration similar to that of the ripe eggs. A special stickiness of the oocytes, as reported for *Par.* by Lindahl and Holter (1941), was not found in the *Ps.* material.

The oxygen consumption figures obtained for oocytes, under-ripe eggs and over-ripe eggs are given in Table III, where, for comparison, the average value for the ripe eggs (calculated from Table II) is also included. The number of experiments on the different cell types is, however, too small to permit of any comparison between the two *Ps.* phenotypes, as was possible in the case of the more abundant material on ripe eggs.

TABLE III

Average rate (r) of oxygen consumption of oocytes, under-ripe eggs and over-ripe eggs of Psammechinus 230 minutes after removal from ovary

n = number of diver experiments, each of which was performed on cell material from different females.

Cell type	r (µl./cell/hour)	σ	ϵ	n
Oocytes	0.66×10^{-4}	0.13	0.04	11
Under-ripe eggs	0.56×10^{-4}	0.17	0.06	10
Ripe eggs	0.51×10^{-4}	0.16	0.02	64
Over-ripe eggs	0.45×10^{-4}	0.09	0.04	7

Obviously there is a gradual, though slight, decrease in oxygen consumption on advancing cell maturation (all differences are statistically *well* established except that between under-ripe and ripe eggs, which is probable but not fully secured).

Although the findings on the *Ps.* material are thus not fully in accordance with those of Lindahl and Holter (1941) on *Par.* they, nevertheless, principally show a lower respiration in the ripe egg in comparison with that in the oocyte. This question will be discussed more extensively later on (see 4). On the other hand, the statement of Boell and co-workers (1940) that the ripe eggs of *Arbacia* consume more oxygen than the immature eggs (oocytes) contradicts these findings. (Possibly the oocytes used in their experiments had been kept for an especially long time in sea water before diver measurements began.)

The relatively low *Ps.* oocyte respiration in comparison with that of the *Par.* oocyte may possibly be connected with some sort of species-specific metabolic change caused by the contact with sea water when the cell is shed. In order to estimate the oocyte respiration inside the ovary an attempt was made to obtain cells immersed in ovarial fluid by means of introducing the tip of a braking micropipette through the ovary wall into the cavity. The habitus of the ovary, which made it very difficult to remove the coelomic fluid from the surface of the ovary, together with its great fragility, made it extremely difficult to draw out pure ovarial content. Two additional factors invalidated the reliability of the diver measurements, viz. first, the dense packing of the cells inside the ovary, which tended to overcrowd the diver bottom drop thus giving bad gas exchange conditions and also causing great difficulties when the oocytes were to be picked out from the extracted material, and second, the frequent occurrence of other coelomic cells, such as amoebocytes, etc., which invariably and inseparably accompanied the desired cells. (Cf. similar experiments on *Ast.* oocytes in 3.122, where all these difficulties were mainly absent.) In any case the diver measurements indicated that the initial oocyte respiration in ovarial fluid is of the same order of magnitude as the initial one in sea water.

3.114. *Size of cells*

The two *Ps.* phenotypic varieties, the *Z*- and *S-forms,* were previously (3.112.2) compared as to the oxygen consumption of the ripe eggs. No significant difference could be established. An attempt was then made to correlate the great biological variability of the oxygen consumption to the size of the eggs. No correlation could, however, be established between these two entities. If the amount of protoplasmic material has any influence on the rate of oxygen consumption, as might have *a priori* been expected, this influence is obscured by other experimentally uncontrolled factors governing the metabolic rate and the size.

TABLE IV

Diameter (d) and volume (v) of ripe eggs from the two Psammechinus phenotypes

n = number of females examined. At least 20 eggs from every female were measured

Phenotype	d (μ)	σ	ϵ	n	Difference in d (μ)	v (μl.)
Z-form	94.6	3.5	1.1	11	9.1	4.43×10^{-4}
S-form	103.7	5.1	0.7	49	($\epsilon_{\text{diff.}} = 1.3$)	5.84×10^{-4}

On the other hand distinct differences in size between the ripe eggs of the two forms definitely exist, as can be seen from Table IV. The significance of the difference is established by the fact that it is 7 times as big as its standard error. Similar results have been gained by Lindahl and Runnström (1929) (*Z*-eggs $d = 98.3\,\mu$; *S*-eggs $d = 114.8\,\mu$).

The size of the eggs is not a given constant. Thus, for instance, the *Z-form* eggs were found to be smaller in 1946 than in 1947 ($d = 87.5\,\mu$ and $d = 94.6\,\mu$, respectively) and Lindahl and Runnström (1929) state $d = 98.3\,\mu$ for eggs of animals from the same district in the late Twenties. Moreover, geographical dissimilarities also seem to exist: Thus Laser and Rothschild (1939) give $d = 103.3\,\mu$ for a Millport material and Hobson (1932) gives a volume corresponding to $d = 118.4\,\mu$ for a Plymouth material. These two materials are no doubt of *Z-form,* for which the figures $d = 87.5$–$98.3\,\mu$ were found at the Swedish West Coast (see above). Hydrographically caused nutritional differences are probably the chief reason for these dissimilarities.

Because of the simultaneous existence of full size oocytes and a variety of small ones, which are not fully mature, it is rather difficult to get a reliable figure for the oocyte diameter. However, the oocytes seem, on an average, to be smaller than the eggs. This is supported by all measurements on material with apparently homogeneous oocytes, especially in the cases in which the oocyte percentage is low. For example a material with an average egg diameter of 96.8 μ showed an average oocyte diameter of 88.8 μ. These findings are in concordance with the results on *Ast.* (see 3.123), where the oocytes are quite homogeneous in size and where the eggs are bigger than the oocytes. Similar results are reported by Lindahl and Holter (1941) in *Par.*

It may be noted, first, that in *Ast.* the (primary) oocytes respire at a much lower rate than the eggs (or secondary oocytes), second, that in *Ps.* the oocyte respiration is instead slightly higher than the egg respiration, and third, according to Lindahl and Holter (1941), that in *Par.* the oocytes respire at a much higher rate than do the eggs. Thus the respiration cannot be correlated to the cell size and furthermore, as the amount of protoplasm will always be nearly the same in oocytes and eggs (though the degree of hydration may differ), it naturally cannot be correlated to the content of organic matter.

3.12. *Asterias*

3.121. *Cell material*

For *Asterias forbesii* Costello (1935) states that the nuclear membrane of the oocytes begins to disappear after about 10 minutes in sea water (19–26° C.) and that the first polar body is separated in about 60 minutes. After another 20 minutes the second polar body appears. Cytoplasmic maturity seems to be attained rather rapidly and to be connected with the diffusion of the nuclear sap into the cytoplasm. Chambers and Chambers (1940) confirm the maturation time schedule.

This scheme obviously also holds for the *Ast.* material of this investigation. When taken from the ovary the cells are, without exception, oocytes. (Recent authors agree that on natural spawning the cells are in a state of broken down germinal vesicle, *i.e.* primary oocytes with initiated first meiosis; cf. Hörstadius, 1939, and Runnström, 1944.) Within 30–40 minutes after being placed in sea water (18° C.) they usually show broken down nuclear membranes in over 90 per cent of the cells. At 60 minutes the first polar body can usually be seen and at 120 minutes often two (or three) are formed. The polar bodies are frequently dissimilar in size.

The natural stimulus for the oocyte development is the sea water (cf., *e.g.*, Hörstadius, 1939). This is clearly shown by the experiments with oocytes in ovarial fluid (3.122), where the meiosis was delayed for several hours. Small contaminations with sea water (or coelomic fluid), however, caused the seminal vesicle to break down rapidly.

After a few hours in sea water the cell material mainly consisted of eggs and a certain small percentage of primary oocytes. In addition, there were a number of secondary oocytes. These, however, were rather hard to distinguish from the eggs because they look exactly the same as the eggs. The only difference lies in the number of polar bodies, and this is extremely difficult to determine when picking out the cells with a braking pipette. Thus the material referred to as "eggs" in the diver experiments, may consist of a mixture of secondary oocytes and eggs. However, the respiration of these two kinds of cells seems to differ only very slightly, since the "egg" respiration agrees very well with that of the young fertilized egg (cf. 3.122). When taken out of the diver after the measurements and then more closely examined under the microscope most of the cells proved to be true eggs. Nevertheless the second meiosis might, of course, have taken place inside the diver during the oxygen consumption measurements.

Any noticeable break in respiration rate could, however, never be observed during diver measurements. Usually the "eggs" were picked out for the diver charge during the second hour in sea water.

The statement of Loeb and Wasteneys (1912) on unfertilized *Asterias forbesii* eggs, that these rapidly die if their oxidative processes are not suppressed, may also be true for the present material. In fact after some 24 hours a "dark" cytolysis is very common. For the diver measurements, which were mostly concluded within 4 hours after the removal from the ovary, this obstacle seemed to be without significance.

For the diver measurements on primary oocytes, such ones as have been mentioned before and which pass quickly over to eggs could not be used. Being a very homogeneous material they would have been very suitable, but measurements could hardly have started before they were already transformed. Thus for the diver experiments only such oocytes as remain as oocytes after some hours in sea water could be utilized. These cells have exactly the same appearance and size as the rapidly converting oocytes. The fact that they remain unconverted seems to point to some sort of immaturity. This might be the case, but only to a certain extent, for the majority of these eggs do actually convert later on, *i.e.* within some few hours after they have been taken out of the diver again for purposes of control. Thus one might be justified in thinking that the figures obtained on this material would not differ very much from those on rapidly converting oocytes, could they have been obtained. This view is supported by the fact that the obtained consumption figures are the same, irrespective of when the future conversion takes place and by the fact that it is possible to correct the obtained values for the oxygen consumption of such oocytes as have their germinal vesicle broken down when inside the diver (by applying the consumption value for eggs). Moreover, experiments with oocytes in ovarial fluid (in this case mostly "rapid" oocytes must be present) show consumption figures of the same order of magnitude as oocytes in sea water but not as eggs in sea water.

Only such oocyte experiments were accepted as, when examined immediately after the completion of the diver measurements, show no or very few oocytes with broken down germinal vesicle. In the latter case a correction could be applied, if the number of converted oocytes was low.

Control experiments on fertilizability were always performed. They proved that the fertilization percentage was always remarkably low, rarely over 50 per cent and often around 20 per cent. This may partly be due to a lowered mobility on the part of the sperms (reason unknown) and partly to improper sea water conditions (see 2.2). Earlier investigators have stated that 100 per cent fertilization can only occur in connection with natural spawning, artificial fertilization always giving a poor yield.

It may be noted that depriving the *Ast.* cells of their jelly hull has as negligible an effect on the oxygen consumption figure as has the same procedure on *Ps.* cells. Thus an experiment with three parallel divers on naked and on coated *Ast.* eggs from the same female gave the average consumption figures of 2.31 and 2.39×10^{-4} μl. per egg and hour, respectively.

3.122. *Respiration of oocytes and eggs*

Even in *Ast.* cells there is a decreasing oxygen consumption rate as has already been described for ripe *Ps.* eggs (cf. 3.112.1). The rate of decrease is not so steep, however, as in *Ps.* Thus to obtain comparable figures, it ought to be possible to use measurements nearer the point of removal from the ovary than in *Ps.* Nevertheless, the transformation of oocytes into eggs described in the preceding chapter makes early measurements impossible. In the second hour after removal this transformation is practically completed. It is thus most convenient to start evaluating measurements about 120 minutes after removal. The time span of the evaluated measurements has, in the case of the *Ast.* experiments also been about 2 hours. This puts the average point of time used for comparison between different experiments (cf. 3.112.2) to 180 minutes after cell removal from the ovary. The same point of time has been used for both oocytes and eggs.

In order to investigate the cell respiration inside the ovary, experiments on cells in ovarial fluid were carried out in complete accordance with those on *Ps.* material (cf. 3.113). The difficulties met with in *Ps.* were not at all present in *Ast.* Firstly, the ovarial cells are, without exception, oocytes which make any secondary selection unnecessary. Secondly, the *Ast.* cells are not so densely packed in the ovary, and amoebocytes, etc., are far fewer. Moreover, the form and comparative strength of the ovary makes it easier to free it from sea water or coelomic fluid on the outside. These experiments were started, however, so late in the spawning season that but few can be recorded here. They merely show a general oxygen consumption similar to that of oocytes in sea water.

The results on oocytes and eggs in sea water and on oocytes in ovarial fluid are given in Table V.

Perhaps it is justifiable to think that the results reported in this chapter indicate that the oocytes inside the ovary have a relatively high oxygen consumption velocity owing to the external supply of metabolically utilizable substrate from the ovary.

TABLE V

Average rate (r) of oxygen consumption of oocytes and eggs of Asterias 180 minutes after removal from ovary

n = number of diver experiments, each of which was performed on cell material from different females.

Cell type	r (μl./cell/hour)	σ	ϵ	n
Eggs in sea water	2.50×10^{-4}	0.33	0.08	18
Oocytes in sea water	1.15×10^{-4}	0.28	0.09	10
Oocytes in ovarial fluid	1.36×10^{-4}	—	—	2

When shed in sea water (or taken out of the ovary) and thus cut off from this supply the rate gradually drops. On transformation into secondary oocytes the rate is considerably raised, but even in this state there is a gradually diminishing oxygen consumption rate.

The difference between eggs and oocytes in sea water is unquestionable (difference = 1.35; $\epsilon_{\text{diff.}} = 0.12$). Tang (1931) using Warburg technique (23° C.) arrived at a considerably lower respiration figure for *Asterias* eggs, viz. 1.14×10^{-4} μl./egg and hour (converted to be comparable to the figure in the present investigation), than that obtained in this investigation. Possibly Tang used another species (species not stated; *forbesii*(?), Woods Hole). Tang's value of the size of the egg ($d = 160\,\mu$) does not, however, differ appreciably from that of *Ast.* (cf. 3.123). It might therefore be natural to conclude that the two consumption values ought to have agreed. The rapid shaking rate of the Warburg vessels in Tang's experiments might probably have caused damage to the eggs. Moreover, Tang could not find any difference in the rate of respiration between eggs and oocytes. Boell and co-workers (1940) using diver technique were also unable to state any difference between eggs and oocytes. The close similarity between their experimental conditions and those of the present investigation makes their results quite inexplicable. (Boell and co-workers have hitherto only published their results as a preliminary note.) On the other hand Brooks (1943) in Warburg experiments on *Asterias forbesii* finds a lower oxygen consumption in oocytes than in eggs.

3.123. *Size of cells*

The size of the *Ast.* egg is given in Table VI. Just as in *Ps.* (cf. 3.114) and in *Par.* (Lindahl and Holter, 1941) the oocytes are slightly smaller than the eggs, as can be seen from Table VII. This difference in volume is, however, definitely too small to account for the found difference in oxygen consumption.

The eggs from the same ovary are often more constant in size in *Ast.* than in *Ps.* The average relative difference (calculated on eggs from 10 females) from the mean diameter were 2.9 per cent ($n = 200$) for *Ast.* and 3.6 per cent ($n = 200$) for *Ps.* eggs.

TABLE VI

Diameter (d) and volume (v) of Asterias eggs
n = number of females examined.
At least 20 eggs from every female were measured.

d (μ)	σ	ϵ	n	v (μl.)
169.1	5.9	1.4	18	25.2×10^{-4}

TABLE VII

Comparison between cell diameters of Asterias oocytes and eggs
Each figure represents the mean of 20 measurements

Female No.	Oocytes	Eggs	$\frac{\text{Eggs}}{\text{Oocytes}}$
1	149.9μ	157.4μ	1.05
2	151.9	157.8	1.04
3	176.0	180.4	1.03
4	156.6	163.1	1.04
5	165.6	169.9	1.03

3.2. *Respiration before and after fertilization*

3.21. *Psammechinus*

In order to follow the oxygen consumption changes induced by activation an attempt was made to perform the fertilization inside the diver during actual measurements. A diver charge type with a diver neck wall drop containing the spermatozoa in sea water was employed (for details cf. "Diver charge Type II" in Borei, 1948). The spermatozoa, however, always crept over the dividing glass surface and gradually reached the eggs before the neck wall drop and the neck seal II were purposely brought together. This premature fertilization was apparently facilitated by the condensed moisture that always prevails on the inside glass surface of the diver. To measure the respiration of the young fertilized egg one is thus obliged to use cells that are fertilized outside the diver. In this case one must be satisfied if the first point of measurement can be obtained at about 40 minutes after fertilization, because charging the diver etc. takes some time.

After the instant rise in oxygen consumption after fertilization, an exponentially increasing part of the respiration curve (Gray, 1926) starts. For the first hours this increase is still quite moderate, thus leaving the curve rather flat. During this time the curve is especially suitable for obtaining comparative values from different egg material. In the actual experiments of this investigation the first evaluated measurement was chosen to be about 60 minutes after fertilization and the last at 180 minutes, thus fixing the point of time for comparison between the different experiments to about 2 hours after fertilization. The results are given in Table VIII.

TABLE VIII

Average rate (r) of oxygen consumption of fertilized eggs of Psammechinus 120 minutes after fertilization

n = number of diver experiments, each of which was performed on cell material from different females.

r (μl./embryo/hour)	σ	ϵ	n
1.84×10^{-4}	0.31	0.06	30

No difference between the two *Ps.* phenotypes could be found (*Ps. Z-form* 1.81×10^{-4} μl., $n = 9$; *Ps. S-form* 1.85×10^{-4} μl., $n = 21$).

Comparing the respiration value of the fertilized egg (Table VIII) with that of the ripe unfertilized egg (Table II) one finds a ratio of 3.6. With *Ps.* eggs Shearer (1922b) using a Barcroft differential manometer technique (14.5° C.) previously found a ratio of 5.7. His measurements on fertilized eggs fall within 30–60 minutes after fertilization. In the case of the unfertilized eggs one cannot judge how long a time elapsed between the removal from the ovary until the measurements were made. This point is certainly of great importance. The earlier the measurements are made the higher the consumption rate of the unfertilized egg will be (cf. Fig. 2). And consequently the higher the rate of the unfertilized egg the smaller the ratio between the oxygen consumptions of the fertilized and unfertilized egg will probably be. In this connection it might be mentioned that the influence of the respiration of the unfertilized egg (at the moment of fertilization) on the future respiration of the fertilized egg is unknown. It is quite possible that a high respiration of the unfertilized egg at the point of fertilization, *i.e.* a still high content of substrate utilizable in the unfertilized state, may be reflected in a relatively higher respiration of the fertilized egg, at least during the first hours.

The value of Borei (1934) on fertilized eggs cannot be used for comparison in this connection because it was referred to the volume of the egg including the fertilization membrane. Owing to the very variable size of this membrane the actual number of eggs in these experiments cannot be calculated.

On material from other sea-urchin species a variety of ratios have been obtained. Only a few will be quoted here. They are mainly taken from the investigations and reviews of Whitaker (1933), Ballentine (1940) and Lindahl and Holter (1941):

Arbacia punctulata	2.6, 3.9, 4.4, 4.5 5.0, 5.0, 5.0, 5.3
Arbacia pustolosa	6.0
Psammechinus microtuberculatus	3.8
Paracentrotus lividus	3.1, 6.0

The cited material is very heterogeneous, representing very different techniques of measurement (such as Winkler titration, Warburg measurements and Cartesian diver experiments) and very differing temperature conditions (15–26° C.). Moreover the actual time after removal from the ovary is never stated for the unfertilized eggs (the most reasonable assumption being that a few hours have elapsed) and in the case of the fertilized eggs the time after fertilization is sometimes uncertain or completely omitted. Thus the significance of the figures is plainly qualitative. They only show that the oxygen consumption of the unfertilized eggs some few hours after their removal from the ovary is considerably lower than that of the fertilized eggs during the first hours after fertilization.

Gray (1926) found that in *Ps.* the rise of oxygen consumption after fertilization followed an exponential curve. Lindahl and Öhman (1938) working on *Par.* were able to confirm this, and Lindahl (1939) established the S-shaped curve characteristic for the oxygen consumption until hatching. After this there is a sudden steep rise associated with the onset of further growth and morphological modifications.

This shape of the respiration curve after fertilization has been perfectly confirmed in this investigation by diver experiments, which are presented in Figure 3. For comparison the curves of Gray, Lindahl and Borei have been plotted on a relative scale (putting the values 30 minutes after fertilization alike) in the small right hand bottom graph. To complete the survey the curve of the correspondingly run diver with unfertilized eggs has also been plotted in the main graph. In this the times of the first mitoses (judged from microscopically observed parallel cultures) and those of ciliation, hatching etc. have also been indicated. It must be observed that the time which elapses before the hatching of the embryos is somewhat longer inside the diver than in parallel ordinary cultures, viz. 9 and 8.5 hours, respectively (cf. 3.3). This delay is always observed and must be ascribed to the relatively dense packing inside the diver. If the embryos, after hatching, are washed out of the diver and cultured in the ordinary way they develop into perfectly normal plutei.

Zeuthen (1947b) working with refined diver technique on *Ps.* (16° C.) was able to show that the primary exponential oxygen consumption curve has waves superimposed upon it. The minima of these waves correspond to the first cleavages (telophase) of the egg. His main graph must, however, be incorrect as to the time position of the cleavages, *e.g.* in the main graph the fifth mitoses is indicated at 5 hours after fertilization, whereas it correctly occurs at about 3 hours and 20 minutes. Assuming, however, that in his small graph the time mark between the fourth and fifth mitoses stands for 3 hours after fertilization the values are in concordance with known cleavage rates. (In Figure 3 of the present paper the first cleavages are marked in their right position on the curve). Moreover, in order to get an entire graphical scheme of the oxygen consumption of the reproduction cell of the female sea-urchin, Zeuthen has completed his *Ps.* curve with the results of Lindahl and Holter (1941) on oocytes and unfertilized eggs of *Par.*, and with

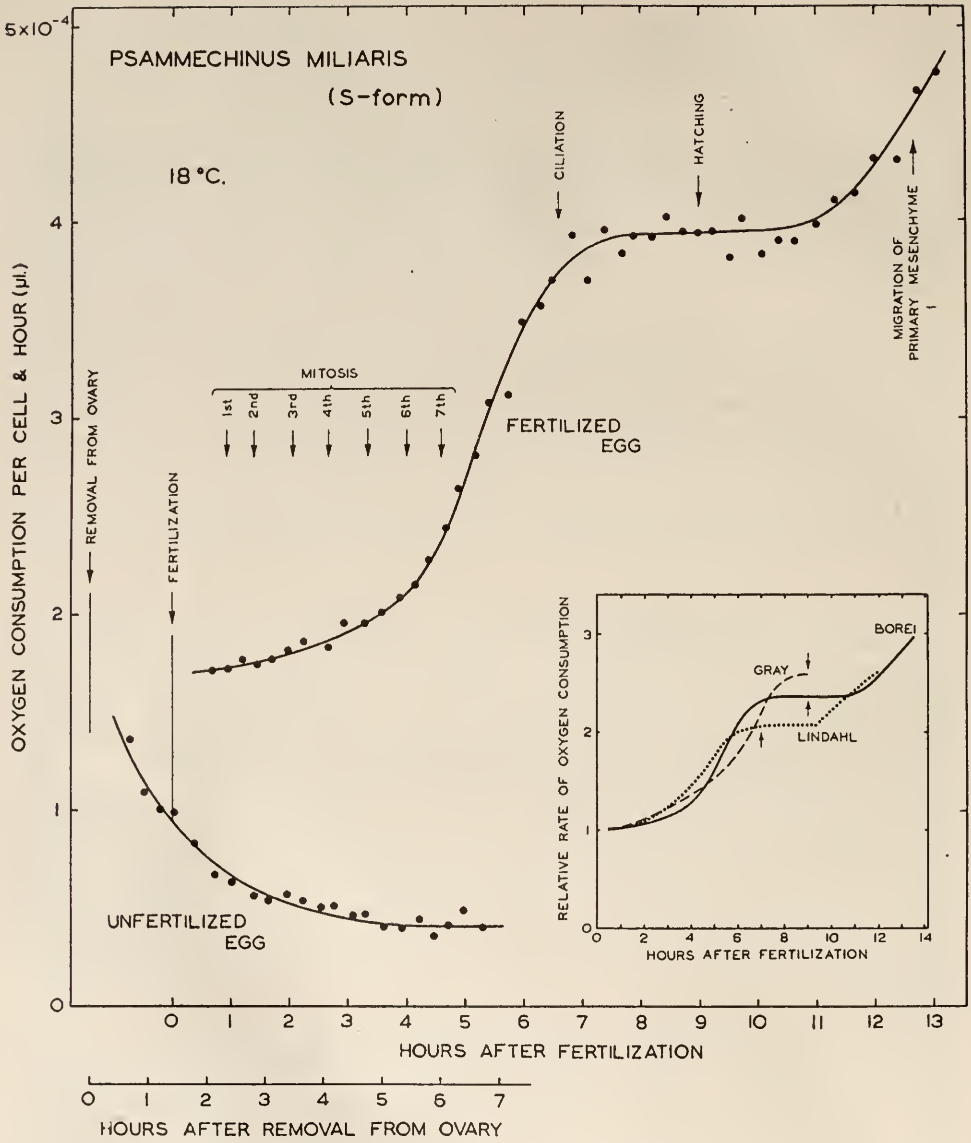

FIGURE 3. Oxygen consumption of *Psammechinus* eggs before and after fertilization.

The values are obtained from parallel divers, one with unfertilized, the other with fertilized eggs. For comparison the results of Gray (1926) on *Psammechinus miliaris* (17° C.) and of Lindahl (1939) on *Paracentrotus lividus* (22° C.) together with those of this investigation (18° C.) are plotted on a relative scale (extrapolated values at 30 minutes after fertilization = 100) in the small right hand bottom graph. In this graph the times of hatching are indicated by arrows. In the main graph the position of the telophase (appearance of cleavage furrow) of the earlier mitoses is indicated.

the results of Lindahl (1939) on fertilized eggs of *Par.* From the results on *Ps.* oocytes and unfertilized *Ps.* eggs presented in this paper (cf. 3.112 and 3.113) it is apparent that the respiration of the *Ps.* and *Par.* cells differs. Thus results from the two species must not be represented as a continuation in one and the same curve.

As has been previously mentioned it has not been possible to use the diver technique to measure the respiration until 40 minutes after the fertilization. Before this time there is said, however, to be a higher oxygen consumption than at any time during the next few hours. This was first indicated in measurements of Shearer (1922a) on *Psammechinus microtuberculatus* and later studied in detail by Runnström (1933) and by Laser and Rothschild (1939) working on *Par.* and *Ps.*, respectively. Nevertheless the exact shape of this part of the curve is still uncertain. (The slight temporary rise over the unfertilized egg value immediately after fertilization, which is indicated in Zeuthen (1947b) in his main graph, is not drawn in accordance with the findings of the mentioned investigators.) The period between fertilization and the first diver measurements has been left empty in Figure 3, but one must, however, keep in mind that during this period oxygen consumption rates, higher than that at 40 minutes, may have occurred.

3.22. *Asterias*

Comparing the respiration rates of unfertilized and fertilized *Asterias* eggs Loeb and Wasteneys (1912) (Winkler measurements) and Tang (1931) (Warburg technique) found no differences. In Cartesian diver experiments Boell and co-workers (1940) confirmed this. The results in the present paper in no way differ from these results. Here the respiration has been followed, however, for a longer space of time than in the earlier investigations, namely over a period of more than 200 minutes after fertilization, *i.e.* over the first mitosis. A gradual increase in oxygen consumption rate is to be noted during this time, as will be seen from Figure 4 (concerning the cell material, cf. 3.121).

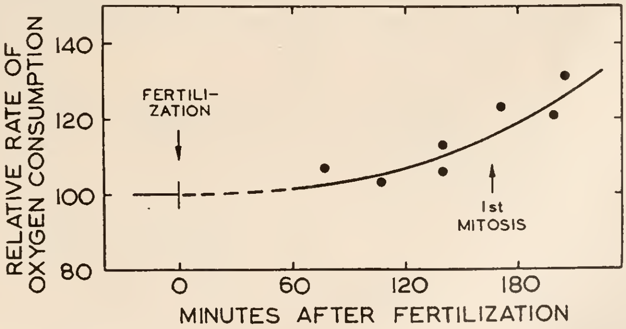

FIGURE 4. Oxygen consumption of *Asterias* eggs before and after fertilization.
The exact position of the first mitosis telophase in relation to the time of fertilization depends on the state of the egg when fertilized (secondary oocyte or egg). In a batch of cells mitosis may thus not occur synchronously.

3.3. *Cleavage rate*

In previous chapters reference has been made to the succession of mitoses and the time of their occurrence after fertilization. Though this cleavage rate in *Ps.* has repeatedly been studied by various authors, it has, for the sake of control, been checked even in this investiga-

TABLE IX

Cleavage rate of fertilized Psammechinus eggs

Concerning right time values in Zeuthen's experiments cf. 3.21

Author	Gray (1926)	Zeuthen (1947b)	Borei (this investig.)
Temperature	17° C.	16° C.	18° C.
Salinity	34–35 $^0/_{00}$	32 $^0/_{00}$	33.2 $^0/_{00}$
	Time after fertilization:		
1st mitosis	67 mins.	—	56 mins.
2nd	100	—	83
3rd	132	—	127
4th	168	165 mins.	160
5th	203	200	~200
6th	238	240	~240
7th	271	(290)	—
Hatching	9 hours	9 hours 30 mins.	8 hours 35 mins.

tion. Found discrepancies were, however, with exception of the first cleavage time, of minor importance as is shown in Table IX.

It should be noted that in this table the values of Borei and Gray were obtained from cultures, whereas Zeuthen's are from observations on dividing eggs inside a diver. In this latter case the hatching time is somewhat delayed. As has been mentioned before (3.21) even in the present investigation a minor delay has been observed in cells when inside a diver.

In Figure 5 the cleavage sequence for *Ps.* and *Ast.* has been represented graphically. For comparison the cleavage rate for the irregular sea-urchin *Echinocardium cordatum* has been plotted in the same figure. All values were obtained during this investigation.

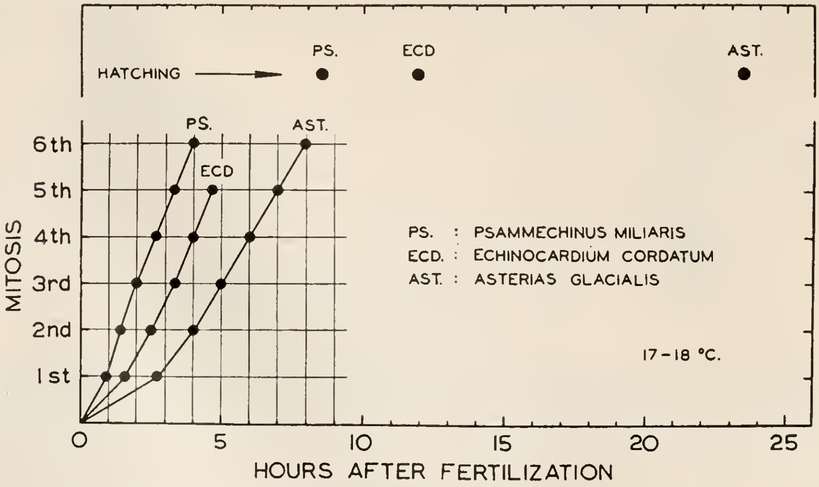

FIGURE 5. Cleavage rate in *Psammechinus, Asterias* and *Echinocardium.*

Values obtained from observations on sparse cultures (200 eggs in 10 ml. sea water). The mitoses are represented from the appearance of the cleavage furrow (teleophase). The position of the first cleavage in *Ast.* is dependent on the state of the egg when fertilized; in the represented sequence the germinal vesicle had just broken down when the sperms were added.

4. General Discussion

Many investigators consider that they have reason to look upon the mature sea-urchin egg as a resting cell between the growing oocyte and the developing fertilized egg. (For literature see Lindahl and Holter, 1941.) The oxygen consumption measurements with diver technique performed by Lindahl and Holter (1941) on *Par.* oocytes, mature unfertilized eggs and fertilized eggs seem to give excellent support to this view. They found that the primary oocytes apparently have a respiration even slightly higher than that of the newly fertilized egg, whereas the unfertilized eggs have a considerably lower oxygen consumption. The authors compare the state of the unfertilized egg with the diapause of certain insect eggs. The investigations of Whitaker (1933) indicate that the low unfertilized egg respiration in comparison with that of the fertilized egg is, among marine invertebrates, peculiar to the sea-urchin group.

The experiments in this paper show, however, that the sea-urchin group is not quite homogeneous in respect to oocyte respiration, as may be seen from Figure 6. Though the oocyte oxygen consumption was measured in principally the same way as in Lindahl and Holter's investigation it was found that *Ps.* oocytes, in contradistinction to those of *Par.,* consumed oxygen at a rather low rate, not differing very much from that of the unfertilized egg.

A new fact, however, is revealed in this investigation concerning the sea-urchin egg respiration, viz. the rapidly decreasing rate of oxygen consumption after the cell has been removed from the ovary (cf. Fig. 2). This declining respiration rapidly and asymptotically approaches a considerably lower value than the initial one (cf. parallels in the endogenous respiration of baker's yeast, kinetically studied by Borei, 1942, and briefly reviewed in 3.112.1). According to these facts the initial rate of oxygen consumption upon egg removal may even be higher than the rate of the fertilized egg during the first hours after activation (cf. Fig. 3). Such a rapidly declining respiration has also been found to be characteristic of the *Ps.* oocytes (3.113).

In all previous investigations the cell material must undoubtedly, though never stated, have been at least some hours old (reckoned from the time of removal from the ovary) when used in consumption measurements. Thus in the present investigation only such *Ps.* and *Ast.* measurements as were obtained from material of that age may be compared with the values of the previous authors. This means that only the measurements on the low level stage which are given in 3.112.2, 3.113 and 3.122 and summarized in Figure 6 may be used for comparison.

If differences now exist, as they probably do, both between the species and between the different kinds of cells within the species, as regards the rapidity with which the low level state is reached, *i.e.,* in steepness of the declining curve, it might in the *Par.* case be that one has hit upon oocytes which show a very slow decrease, whereas in the *Ps.* case the oocytes show a rapid decrease. Under such circumstances the apparent contradictions between *Ps.* and *Par.* oocyte respiration can be understood.

In the case of Boell and co-workers' (1940) findings in *Arbacia* of a lower respiration in the oocytes than in the eggs, it might be either that the oocyte respiration decrease is especially rapid or that the respiration decrease of the eggs is especially slow in that species, or it might be that the time which has elapsed since the removal from the ovary is longer for the oocytes. As no technical points whatsoever are stated in Boell and co-workers' paper it is impossible to draw any conclusions.

It is very interesting to discover that there is general agreement within the sea-urchin group as to a low respiration in the unfertilized egg (when measured some hours after removal from the ovary) in comparison with the respiration of the fertilized cell. (The reviews of Whitaker (1933) and Ballantine (1940) and the results given in 3.21 might be consulted.) Now Loeb and Wasteneys (1912) have found that unfertilized *Asterias* eggs which respire at the same rate as the

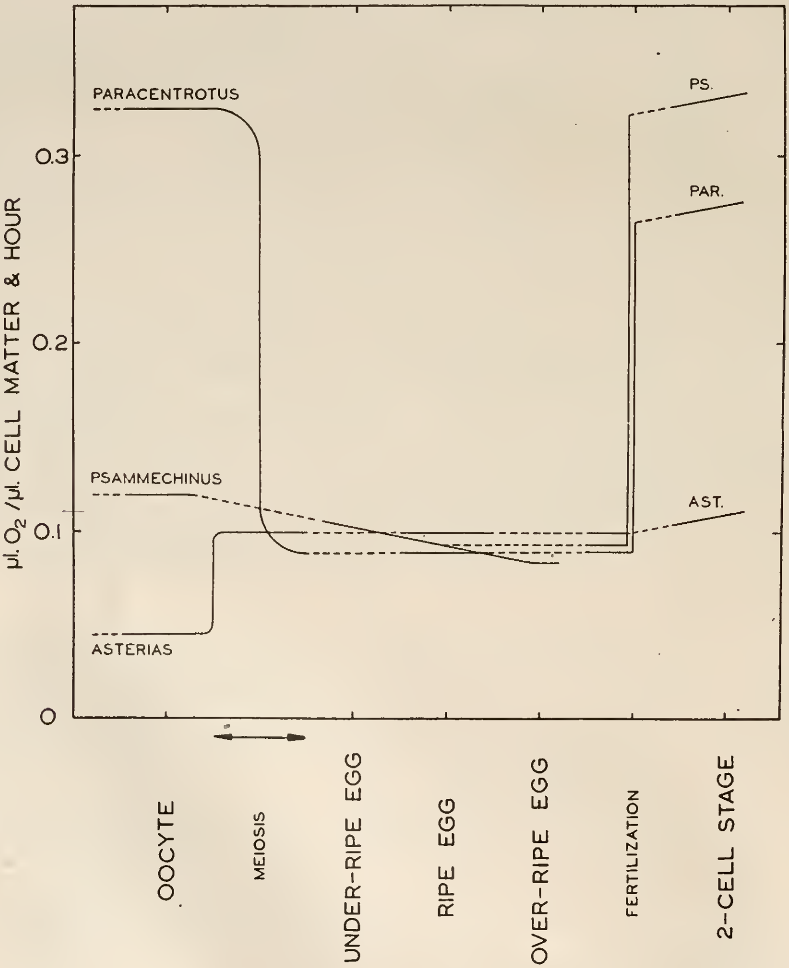

FIGURE 6. Comparison on a cell volume basis between the oxygen consumption of different female reproduction cells from *Psammechinus, Paracentrotus* and *Asterias.*

The results on *Ps.* and *Ast.* are from the present investigation; those on *Par.* from Lindahl and Holter's (1941). In the unfertilized cells the oxygen consumption was always measured several hours after cell removal from the ovary. All rates are uniformly calculated for 18° C. and expressed as if measured at 0° C. and at normal barometric pressure. Average cell volume values used: *Ps.* 5.56×10^{-4} µl., *Par.* 5.75×10^{-4} µl. and *Ast.* 25.2×10^{-4} µl.

fertilized ones, die very rapidly if not kept anaerobically (*i.e.,* if the oxidative processes within the eggs are allowed to continue at an unlimited high level). In view of this and the considerations of other authors one is inclined to look upon the low respiration of the sea-urchin egg as a natural precaution in order to facilitate long life for the shed egg. In this connection one must consider the actual conditions of the localities at time of spawning. The animals there live rather close together and spawning probably sweeps epidemically and simultaneously over the specimens, both male and female, of a given species in a community. Thus a rapid fertilization may be secured, which would mean that the egg respiration would only have time to enter the very first part of the declining curve. It is possible that eggs, which accidentally are not fertilized at once, are preserved for future activation through this low-level mechanism, which thus promotes the reproduction possibilities of the group. On the other hand, however, the similar declining curve of the oocyte respiration will probably have no biological significance whatsoever because, as is generally agreed, the sea-urchin oocytes are never shed.

The biochemical mechanism that causes the differences in respiration between fertilized and unfertilized sea-urchin eggs, as studied by Runnström (1930, 1933, 1935), Örström (1932), Korr (1937) and others, is likely to be characterized in the unfertilized egg by a block in the chain of carriers which, in the fertilized egg, mediates the oxidation of the substrates. This chain of carriers is supposed to include the cytochrome-system. Runnström considered that the oxidase was unsaturated with its substrate, and later investigations have furthered the view that substrates, dehydrogenases and oxidase are kept apart in the unfertilized egg. It is remarkable that the respiration of the unfertilized egg is insensitive to inhibitors affecting the cytochrome-system, whereas the respiration of the fertilized egg is very sensitive. In all these investigations it has been pointed out that the respiration of the unfertilized egg is low in comparison with that of the fertilized egg. This means that the studies have been performed on unfertilized eggs, in which the declining respiration part has already reached a low level. As the initial respiration of the unfertilized egg (when just removed from the ovary) can be even higher than that of the fertilized egg, the question therefore arises as to the relation between the rapidly declining (monomolecular) respiration part of the unfertilized egg and the respiration of the fertilized egg. Whether or not the oxidase is as important for this part of the respiration of the unfertilized egg as it is for the fertilized egg can probably be settled if inhibition experiments are performed as early as possible after the eggs have been removed from the ovary, *i.e.* when the declining respiration is still prominent.

The oxygen consumption figures in the three stages of ripeness of the unfertilized *Ps.* egg reveal that the respiration value slightly decreases with growing ripeness (cf. 3.113 and Fig. 6). It has not been possible to establish any difference in the early declining respiration of these stages. The material is, however, too small for convincing interpretations. (It should be kept in mind that the respiration of unfertilized sea-urchin eggs when kept for a long time in sea water undergoes a change, thus becoming more like that of the fertilized egg.)

In the case of the *Ast.* egg it has been found that the respiration is lower in the primary oocyte than in either the secondary oocyte or the unfertilized egg (cf. 3.122 and Fig. 6), but this finding is in contradiction to earlier ones (Tang, 1931, and Boell and co-workers, 1940; cf., however, Brooks, 1943). As the eggs on natural spawning are shed with broken down germinal vesicle, *i.e.* as secondary oocytes or as eggs (cf. Runnström, 1944), the jump in respiration rate between primary and secondary oocytes does not occur outside the ovary. The respiration after fertilization proceeds (as has been reported by several previous investigators) at much the same level as before. As the decreasing part of the respiration of the cells after their removal from the ovary is not very marked in this material, the rate of respiration of the fertilized eggs may not, under natural conditions, differ very much from that found in this investigation (3.22), even if the fertilization takes place very soon after spawning. Thus if the rates of respiration of the fertilized sea-urchin eggs and the fertilized starfish eggs are compared on a cell volume basis (see Fig. 6), a very much lower respiration in the case of the starfish will be found. Whitaker (1933) has argued that the oxygen consumption of fertilized marine animal eggs from several invertebrate phyla and even of other developing cells, when compared on a cell volume basis, will show a remarkable consistency, whereas the respiration values of the unfertilized cells are widely scattered. It may be read from Whitaker's discussion, though not stated in these terms, that the respiration of the fertilized eggs is thought to be intimately connected with the work of morphogenesis and with biochemical activities connected with growth, and that in developing cells the amount of oxygen required for these purposes is about equal per volume of cytoplasmic matter. It is stated, however, that big cells, especially yolky eggs respire at a much lower rate. Other factors to be considered will, no doubt, be different degrees of cytoplasmic hydration, inert inclusions in vacuoles, etc., dead protecting or otherwise supporting structures, etc. The measurements by Tang (1931) on *Ast.* egg respiration before and after fertilization gave too low values to fit in Whitaker's scheme. The latter author severely criticizes technical weaknesses in Tang's measurements and leaves them out of his survey. The present investigation has certainly found Tang's values to be notably low (cf. 3.122), but still the fertilized *Ast.* egg respiration is remarkably low in comparison with that of the sea-urchin egg (cf. Fig. 6). It should be borne in mind that the *Ast.* egg has a volume that is about 5 times greater than that of the *Ps.* or *Par.* egg. This fact may probably, in the light of the discussion on the connection between body size and metabolic rate recently put forward by Zeuthen (1947a), be of more importance than considerations concerning supposed morphogenetic work.

To summarize the egg respiratory conditions found in this investigation together with those previously known, the graphical schemes in Figure 7 may serve.

The *Ps.* ripe egg (Fig. 7a) (cf. 3.112.1) has very probably a high respiration level in the ovary; at least it starts with high respiration velocity when brought into sea water. The oxygen consumption rate rapidly decreases and within some hours reaches a low and fairly constant level. The oocyte behaves similarly. At fertilization the rate immediately rises to around the level of the just removed egg, drops slightly and thereafter proceeds after an exponentially increasing curve (cf. 3.21). After natural spawning, fertilization probably occurs very soon, thus leaving the decreasing curve without much importance. Only ripe eggs are fertilizable.

The *Ast.* primary oocyte oxygen consumption rate (Fig. 7b) (cf. 3.122) decreases comparatively slowly in sea water. If the oocyte is ripe it soon starts the first meiosis, thereby increasing its respiration considerably. The second meiosis soon follows and a slow decrease in oxygen consumption continues until fertilization occurs. After fertilization the consumption proceeds as an exponentially increasing curve (cf. 3.22). On fertilization no sudden jump in consumption rate occurs as in *Ps*. When at natural spawning the cell leaves the ovary its nuclear membrane has disappeared. Thus the jump in the rate of respiration on transformation from primary to secondary oocyte has already occurred. Even oocytes may be fertilized, but then the time which elapses before the first mitosis will be longer thus allowing meioses to occur and thereafter the resting sperm nucleus to unite with the egg nucleus.

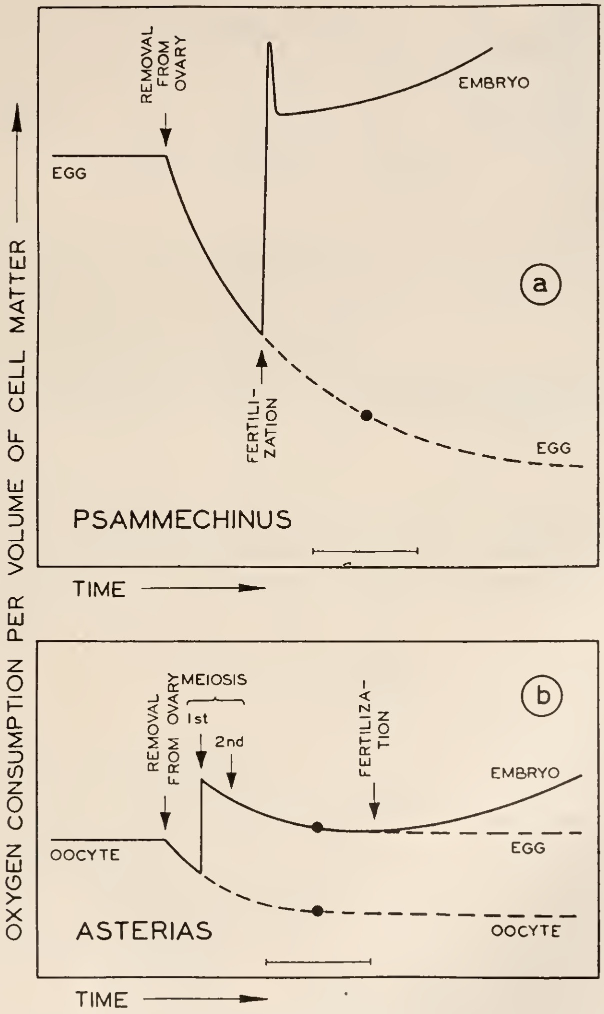

FIGURE 7. Generalized schemes of oxygen consumption per cell volume of reproduction cells of *Psammechinus* and *Asterias*.

Time span of evaluated diver measurements as well as location of comparison value on respiration curve indicated for each cell type.

It is interesting to note that in fertilized eggs of both *Ps.* and *Ast.* the increase in oxygen consumption, which takes place during the first hours of development after fertilization, seems to be of the same exponential type in both species. This will be seen from Figure 8, where values from the first 4 hours' development in both species have been plotted on a relative scale putting the values at 40 minutes after fertilization = 100.

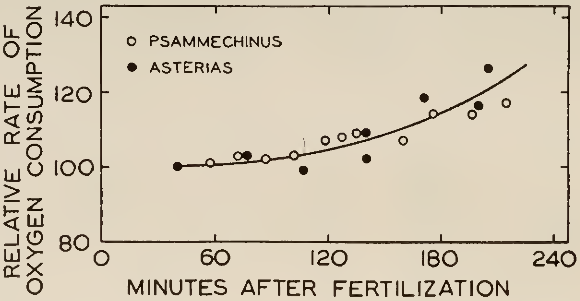

FIGURE 8. Relative oxygen consumption of fertilized *Psammechinus* and *Asterias* eggs. Values at 40 minutes after fertilization put = 100.

5. SUMMARY

In Cartesian diver experiments on the oxygen consumption of oocytes, unfertilized eggs and fertilized eggs from the sea-urchin *Psammechinus miliaris* and the starfish *Asterias glacialis* it was found:

1. The respiration of ripe *Ps.* eggs declines rapidly after they have been removed from the ovary into sea water. Starting at a rate that may exceed that of newly fertilized eggs it has thus, after some hours, attained a comparatively low and fairly constant level. The declining curve on kinetical analysis proves to be composed of a monomolecular and a constant part. The respiration curve of *Ps.* oocytes is of a similar type. In *Ast.* oocytes and eggs the respiratory decrease, though present, is not so prominent as in *Ps.* cells (3.112.1, 3.113, 3.122, Fig. 2).
2. Though there is a real difference in size between the eggs of the two *Ps.* phenotypes (the littoral *Z-form* and the *S-form* of the depths) no difference is found in the rate of respiration (3.112.2, 3.114).
3. Measurements on *Ps.* oocytes and eggs some hours after removal from the ovary show that the oocytes have only a slightly higher respiration than the eggs. The earlier investigations (Lindahl and Holter, 1941) on *Paracentrotus lividus* eggs showed that these oocytes maintain a rate of respiration even higher than that of the newly fertilized egg. The findings in *Par.* might be ascribed to a slow respiration decrease in the oocytes, whereas the decrease is more rapid in the eggs. In *Ps.* the decrease is about equal in oocytes and eggs (3.112.2, 3.113, 4, Fig. 6).
4. In *Ast.* the primary oocytes respire at a much lower rate than do the secondary ones or the eggs (3.122, 4, Fig. 6).
5. In *Ps.* there is a gradual slight decrease in egg respiration with advancing cytoplasmic maturity (3.113).
6. In both *Ps.* and *Ast.* the respiration of oocytes in ovarial fluid seems to be of the same order of magnitude as that of oocytes in sea water (3.113, 3.122).

7. The shape of the respiration curve in *Ps.* after fertilization is in full concordance with earlier results obtained with different techniques by Gray (1926) and Lindahl (1939) (3.21, Fig. 3).

8. The value of the rise in respiration, that occurs in sea-urchin eggs on fertilization, may entirely depend on where on the slope of the decreasing egg respiration curve fertilization occurs. (This rise is characteristic for sea-urchin eggs and has repeatedly been found by earlier investigators.) It is thought that on natural spawning the rise is rather feebly marked because of early fertilization, and that correspondingly the low level respiration of the unfertilized egg may not be reached (3.21, 4, Figs. 3 and 7).

9. In *Ast.* there is no immediate rise in respiration after fertilization, but there is a gradual rise which exactly resembles the exponential increase in newly fertilized sea-urchin eggs (after the first sudden increase has passed). The rise from the oocyte respiration level to that of the egg will, under natural conditions, not occur outside the ovary, as the cells are shed with broken down nuclear membranes (3.22, 4, Figs. 4, 7 and 8).

Cleavage rates are given up to the sixth mitosis for *Ps.*, *Ast.* and *Echinocardium cordatum;* hatching time is noted (3.3, Fig. 5).

It is discussed whether the decrease in respiration of the unfertilized sea-urchin egg after its removal from the ovary has any possible significance for the biochemical aspects of the sea-urchin egg respiration (4).

If the respiration rates found in this investigation are compared on a cell volume basis it is found that the *Ast.* egg will not fit into the generalized scheme of Whitaker (1933) for marine invertebrate eggs; it is discussed why the *Ast.* egg respiration is so comparatively low (4, Fig. 6).

Acknowledgments: This investigation was carried out during 1946 and 1947 on the Swedish West Coast at the Kristineberg Zoological Station of the Royal Swedish Academy of Science. The author wishes to express his deep gratitude for laboratory facilities and for the great pains taken by the Station Staff in supplying materials. He is much indebted to Professor J. Runnström for encouragement and for keen interest in the subject.

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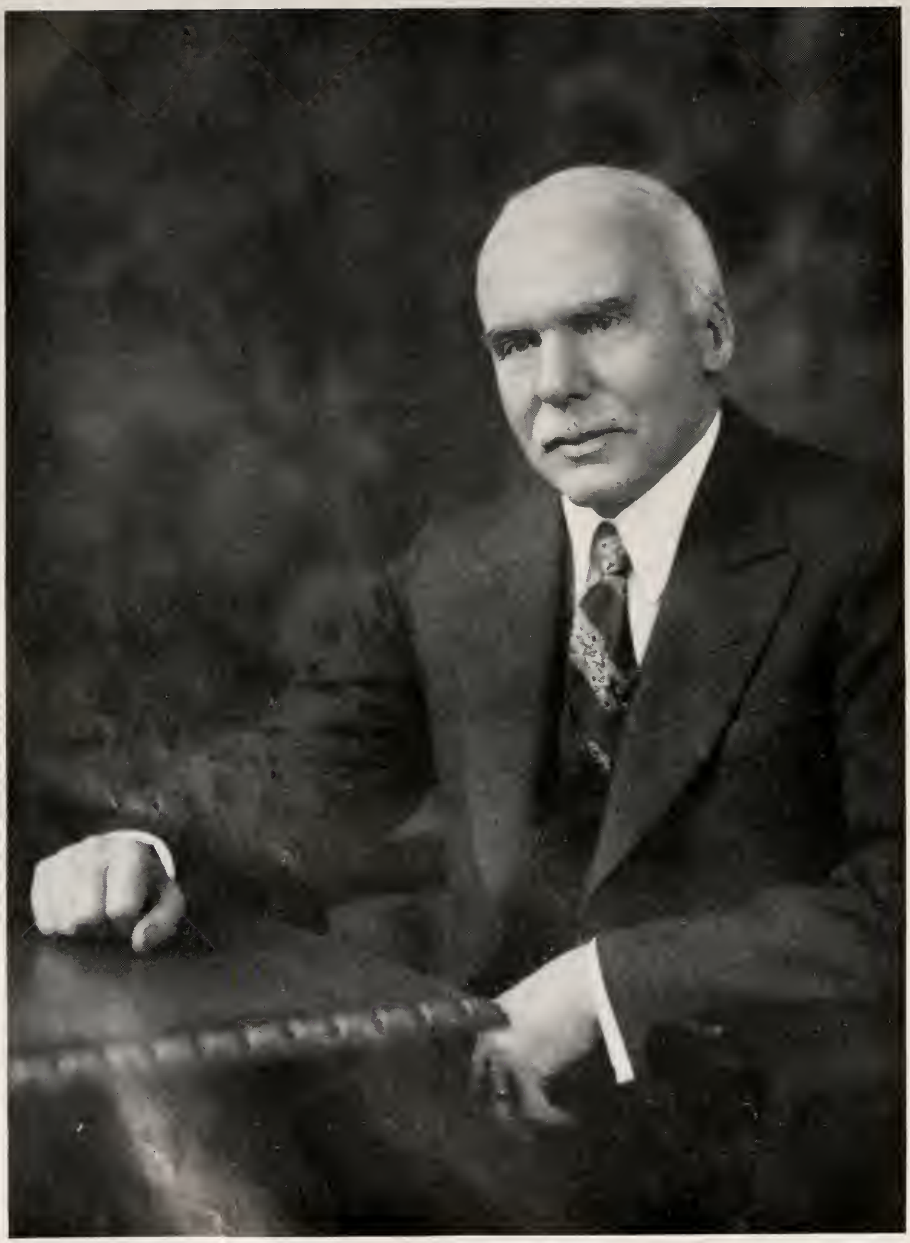

FRANK RATTRAY LILLIE

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ADDRESSES AT THE LILLIE MEMORIAL MEETING WOODS HOLE, AUGUST 11, 1948

B. H. WILLIER, R. G. HARRISON, H. B. BIGELOW, E. G. CONKLIN

FRANK RATTRAY LILLIE

1870–1947

The Work and Accomplishments of Frank R. Lillie at Chicago

It is my privilege and honor tonight to review and appraise the work and accomplishments of Frank Rattray Lillie at the University of Chicago. The year following his graduation in 1891 from the University of Toronto he was fellow in zoology at Clark University, where he began graduate studies under the direction of C. O. Whitman, one of the early leaders in American zoology and the first director and one of the guiding spirits in the early days of the Marine Biological Laboratory. In 1892 the dynamic President Harper lured Whitman, among other prominent scientists, away from Clark University to the newly founded University of Chicago. With affectionate admiration and devotion every student went with Whitman. There two years later (in 1894) Lillie received, at the age of 24, the degree Doctor of Philosophy in Zoölogy. In accord with the trend in embryology of those days, his thesis dealt with the phenomena of cell lineage as it occurs not in a marine species, but in the fresh water clam, *Unio*. Worthy of special mention here is an evaluation of this work in his own words, written only four years ago: "A feature of special interest in the first publication was a discovery I made in studying the cell-lineage of *Unio,* that the behavior of the individual cells was adaptive and that varying sizes, rates and direction of division of cells were directly related to the subsequent events." To him development at any particular time and place is always a special feature directly related to functional need. Herein lies the key to his philosophy of embryology and of biology in general.

Except for a period of six years following the doctorate, his professional life was intimately associated with the University of Chicago from the time of its founding. During this period he was for 5 years (1894–99) instructor of zoölogy at the University of Michigan and for one year (1899–1900) Professor of Biology at Vassar College. In the fall of 1900 he was called back to Chicago as Assistant Professor of Zoölogy, which in 1906 culminated in a professorship of Embryology at the age of thirty-six years. Upon the death of Whitman in 1910, Lillie succeeded him as chairman of the Department of Zoölogy and continued in that position until 1931, a span of 21 years. From 1931 to 1935 he was Dean of the Division of the Biological

Sciences. Concurrently in recognition of his outstanding achievement in research and service to the university and to the science of biology at large he was the Andrew MacLeish Distinguished Service Professor of Embryology, and thereafter held this title emeritus until his death.

Outstanding among his accomplishments as chairman was an effective departmental organization which was characterized by a lack of elaborate administrative machinery and by a policy which encouraged both freedom and initiative for the individual, whether staff or student. In dignity and simplicity he administered without seeming to do so. His was a leadership in which everyone knew his position, responsibility, and opportunity. With the growth of the department, the need for additional research facilities became increasingly great. This need was met to a large extent in 1936 when he and Mrs. Lillie very generously presented to the university The Whitman Laboratory of Experimental Zoölogy. Named in honor of Professor Whitman, the first head of the department, it stands today as a monument to the research ideals of both teacher and student.

The four-year term as Dean of the Division of Biological Sciences began only one year after the clinical departments of a new medical school were set up on the Midway campus. In Dr. Lillie's own words his "special task as dean was to amalgamate the old established pre-clinical departments with the newly established clinical departments and hospitals into a coherent medical school." Through much patience, vision, and understanding he succeeded in uniting the departments of basic sciences and clinical biology into a cooperating group which is unrivalled elsewhere. Today this working union stands as a model of what can be accomplished along these lines. Of this achievement Dr. Lillie was justly proud.

Professor Lillie's influence as a teacher had a far-reaching effect on students. His lectures were always characterized by a masterful precision of organization and conciseness of statement, the force of which was perhaps sometimes not fully effective owing to his soft-spoken and undramatic manner of delivery. The graduate students were not taught in the conventional way. "The student was trained to think by one who directs without seeming to do so, and was attracted first of all to the organization of the seminar and graduate courses in which the results of research, interpretations, and theories were ingeniously knit together around a central theme. Thus, the alert student was able to see how an apparently insignificant detail was concisely and cleverly woven into a concept with significant implications. The student soon learned to judge and evaluate his own performance in the seminars. The example somehow led him to strive for perfection in organization and clear thinking. The young student when he began research was to a large extent thrown upon his own resources. He found out for himself whether he was fitted to be an independent investigator. Once the problem was suggested and the way of approach briefly sketched, the student knew that results were expected. Only when a preliminary result was obtained did the student report to Dr. Lillie, and even then only when he was prepared to make a possible interpretation." (*Anat. Rec.,* 100: 409.)

Although his scientific life was shared almost co-equally between the Marine Biological Laboratory and the University of Chicago and these interests are consequently difficult to separate, I shall confine my remarks only to those discoveries which were made solely at the latter institution. Lillie's studies on the action of sex hormones in foetal life of cattle grew out of attempts to find an explanation of the sterility and partial inversion of the female of two-sexed cattle twins, popularly known for a century or more among cattle breeders as the free-martin. Apparently his attention was first drawn to this peculiar condition in a herd of cattle on the family farm northwest of Chicago near the village of Wheeling, Illinois. The embryological phases as worked out were made possible by the close proximity of the university to the stockyards, in which large numbers of cattle are slaughtered daily. As a student, I remember seeing him garbed immaculately in a white gown and wearing rubber gloves, examining and dissecting pregnant uteri containing young twins which the collector had rushed to his laboratory table in a breathless manner. He found that when the foetal membranes of male and female embryos are fused so that the blood vessels (especially the arteries) of the two are continuous, the female embryo is modified in the male direction. Furthermore, if no blood connection occurs both partners are normal. Without going into the complete evidence, it is sufficient to point out that no detail was overlooked in unravelling how (as he put it) "nature performed a crucial experiment" on the question of the rôle of sex hormones in embryonic differentiation of sex characters. The clear-cut and logical analysis of the free-martin published in 1917 is a model of scientific analysis and probably will stand as his most significant and enduring contribution. This work introduced biologists to the problem of sex hormones at a time when practically nothing was known about the subject. "It did much to initiate an era of intensive research in the field at large and remains as one of the important foundation stones on which a great edifice has since been built in that field." (*Anat. Rec.*, **100**: 408.)

Desiring to test and extend the application of the principles of hormone action thus gained from studies on the free-martin, he immediately initiated an extensive program of research on the isolation of sex hormones and on their rôle in controlling the expression of sex characters. For his own phase of the program he selected the Brown Leghorn fowl, a breed which exhibits what is perhaps one of the most striking examples of sexual dimorphism of plumage color. In collaboration with his research associates (especially Dr. Mary Juhn and Dr. Hsi Wang) some of the fundamental laws governing the action of hormones (estrogens and thyroxin) in the production of color patterns in feathers were discovered. Clues as to the processes involved were found to lie in the feather papilla or germ itself, a specialized derivative of the skin which exhibits many of the characteristics of an embryonic system such as axial organization, organizer-like action, and reaction gradients to hormones. Indeed, the feather papilla is an embryonic system in miniature and remains so thoroughout the life span of the bird. In elucidating these and similar problems Lillie was active almost to the end and, like his old teacher C. O. Whitman, finished on the theme of the underlying mechanisms of plumage color design in birds.

Frank R. Lillie was a part and a great part of what was highest and best in the life of the University of Chicago. His name has been built into the annals by his insight into great and guiding principles as well as by his special achievements and services. He will always remain a part of the University's treasured memories and noblest heritage.

B. H. Willier

Dr. Lillie's Relations to the National Academy of Sciences and the National Research Council

The phase of Dr. Lillie's life assigned to me for consideration has to do with his relations to the National Academy of Sciences and the National Research Council. Now I find that the records of these services are bound up in annual reports and minutes of meetings, material as dry as dust and as soulless as a stone, but there must be more in them than these records would seem to allow, for Lillie himself has said, in an autobiographical memorandum left with the National Academy, that among the offices he has held in various scientific societies he valued most the Presidency of the Academy and the Chairmanship of the Research Council. It is not possible, of course, to separate the character and personality of a man from his aims and achievements, and so we must look behind the published records to get the full story of his life, even as regards his formal relations to the organizations he served.

Lillie was elected to the Academy in 1915, the year before the Research Council was established. There is nothing in the source material available to indicate that he was very active in the Academy during the first few years of his membership. His modesty and observing character must naturally have led him to assume a waiting attitude until he became familiar with its procedures.

In the year 1919, the Society of Zoölogists chose him as one of their first representatives in the Division of Biology and Agriculture of the National Research Council, an assignment that he was to hold for four years. He was immediately made a member of the Executive Committee of the Division, the chairman of which was C. E. McClung, and was appointed chairman of two other committees, one on Coöperation and Coördination, of which I have found no record of activities, and one that must have been especially dear to his heart, on the Marine Biological Laboratory at Woods Hole. This was during the period when the Laboratory was hopeful of greater and better opportunities, when Mr. Crane had already made many of his generous gifts, including the building that now bears his name, but when it still needed a broader basis of support. In several of the annual reports of the Division in this period, a plaintive, but optimistic note is struck in such statements as this: "* * * the division and council have approved the program of the Marine Biological Laboratory and have sought energetically to secure support for this important coöperative institution. As yet this financial aid has not been secured, but it is unthinkable that it should not eventually come." The story of how it did come is told in Lillie's book on the history of the Laboratory, and I shall return to this briefly after consideration of his other accomplishments in the Division of Biology and Agriculture.

While continuing as chairman of the above committees, Lillie was chosen Vice Chairman of the Division for the year 1921–22, and became Chairman the following year. During the latter period, two important projects were brought to fruition. The first was the organization of the Union of American Biological Societies, which was later to assume responsibility for the publication of *Biological Abstracts*. The second was the establishment of the National Research Fellowships in the Biological Sciences through an initial grant of $325,000 from the Rockefeller Foundation. Lillie became Chairman of the Fellowship Board, serving as such until 1931, during the period when its policies and procedures were determined. After 1937, when the fellowships in the physical and the biological sciences were merged into the National Research Fellowships in the Natural Sciences, he served for three years on the joint board.

To return now to the Marine Biological Laboratory: The report of Lillie's committee endorsing its program was formally approved by the Division of Biology and Agriculture and by the Executive Board of the National Research Council. Then through Dr.Vernon Kellogg, Permanent Secretary of the Council, who was also a member of the Executive Committee of the Rockefeller Foundation, the attention of the officers of the Foundation, including the President, who had been a colleague of Lillie's at the University of Chicago, was drawn favorably to the Laboratory. This led, early in 1923, to a conditional grant. By the end of the year the conditions were met, so that the Laboratory could go on with its building plans, with assurance that its endowment would be sufficient for some years to come. The building in which we are now assembled was dedicated in July, 1925.

Lillie's account of these events sounds somewhat naive in view of all the invective that has been hurled at interlocking directorates, but it evidences a wholly honest spirit without fear of criticism for taking advantage of personal and official connections in such a good cause.

Many others were, of course, actively associated in the movement to provide the Laboratory with adequate resources to satisfy the growing demands of biological research in this country. Nevertheless, it was the quiet reasonableness of Lillie's leadership as Director of the Laboratory that carried conviction and inspired confidence in all with whom he had to deal in bringing this undertaking to a happy conclusion.

While the new laboratory was under construction, conditions that were to lead to the germination of a new enterprise were taking shape. One of the persons who became interested in the Laboratory at that time was Wickliffe Rose, President of the General Education Board. Dr. Rose's interests in biology were broad; conversations were held with Lillie, and by 1925 they began to center on oceanography, in particular on the establishment of an oceanographic institution on the East Coast of the United States. It was decided that the best approach to realization of the project would be to present the plan first to the National Academy for study. This was done at the Annual Meeting in 1927, at which a motion was unanimously passed to the effect: "That the President of the Academy be requested to appoint a Committee on Oceanography from the Sections of the Academy concerned to consider the share of the United States of America in a world-wide program of Oceanographic Research and report to the Academy." Lillie's presentation of the case must have been persuasive to secure such prompt action, for the committee was appointed immediately with Lillie as chairman, and after a preliminary meeting, a liberal grant was made by the General Education Board to defray the cost of conferences and special studies on the subject. In particular, Dr. Henry Bigelow, who will no doubt say more about this, was invited to make investigations for the Committee for use in the preparation of this report, which in preliminary form was presented to the Academy at the Autumn Meeting, November, 1929. Closely following this the Woods Hole Oceanographic Institution was incorporated, and in February, 1930, the Rockefeller Foundation made to it a grant, ultimately amounting to three million dollars, for construction and equipment of laboratory and ships and endowment of the Institution. Lillie became its first President, serving until his retirement in 1939. At the same time a grant was made to the Trustees of the Bermuda Biological Station to meet the conditions of a gift from the Colonial Government. These two grants enabled the Station to proceed with its plans for development. In recognition of his services to oceanography the National Academy in 1940 awarded him the Agassiz Medal.

His three main accomplishments of the decade 1920–30 in connection with the Academy and Research Council, i.e., the establishment of the fellowships in the biological sciences, the placing of the Marine Biological Laboratory on a secure financial foundation with greatly increased facilities for work, and the foundation of the Oceanographic Institution, add up to more than most men can look back to in an equal length of time. In promoting these activities, he was either acting as agent for the Academy and Council, or was using quite legitimately the prestige of these organizations to further the purposes of other institutions he had at heart. All of this was done while administering a large department at the University of Chicago, serving as director of the Marine Biological Laboratory and engaging in research of high quality.

Lillie served the Academy and the Research Council in various other ways. He was chairman of the Daniel Giraud Elliot Medal Committee, 1926–29, and of the Murray Fund Committee, which awards the Agassiz medal, 1929–32.

Shortly after the Committee on Problems of Sex was set up in the Division of Medical Sciences with funds for grants-in-aid on a liberal scale, Lillie was appointed a member. He had a few years before, when little was known of sex hormones, completed a remarkable piece of research on the development of the freemartin, the female member of a heterosexual twin-pair in cattle, which is regularly sterile and gives evidence of partial sex inversion. His explanation of this phenomenon gave an entirely new turn to the then prevalent views on sex differentiation and constitutes one of the foundation stones of the imposing edifice that has since been erected in this field. Lillie thus brought to this important committee mature judgment, based on experience, and became one of its most valued members.

In 1933, when our country was in the midst of its most severe economic crisis, the Government turned to the Academy and Research Council for assistance, and the President of the United States appointed by executive order a "Science Advisory Board with authority, acting through the machinery and under the jurisdiction of the National Academy of Sciences and the National Research Council, to appoint committees to deal with specific problems in the various departments." All but two members of the Board were also members of the Academy and Lillie was included in its number. However, a misunderstanding of the responsibilities of the Academy for the appointment of its own committees led to an unfortunate controversy between the administrative officers of these two bodies. The board worked without regard to this and had brought in two long and valuable reports by the time of its discharge in 1935. Nevertheless, the differences in opinion between the officers of the Academy and the Council had been so sharply drawn, that when the time arrived for a change, it seemed to those concerned that the best way out would be to effect a personal union between the presidency of the Academy and the chairmanship of the Research Council. Lillie, because of his personal qualities and administrative skill, was unanimously chosen to fill this dual post. He went about his task with quiet determination, and by the end of his first year of office the two organizations were working so smoothly that he felt he could relinquish the chairmanship, retaining the presidency for a full term of four years. One of the administrative staff writes of this period: "I always thought that through the force of his integrity and high esteem in the scientific world he was able to create in the minds of a number of other opposing members of the Academy a better understanding of the place of the Council in the Academy's structure." Those who succeeded him in two offices owe him a debt of gratitude for his statesmanship.

As President of the Academy, 1935–39, Lillie showed his usual human qualities. There were no spectacular happenings, but real progress was made in several directions, such as the establishment of the Central Purposes Fund through a grant from the Rockefeller Foundation, and the inauguration of the Pilgrim Trust Lectures. The former is a much needed fund for financing conferences, government relations and international projects; the latter, now unfortunately in abeyance for lack of funds, involved an exchange of lecturers between the National Academy and the Royal Society of London.

The subject of my address has necessitated emphasis on Lillie's administrative work, but I should not wish to close by leaving with you the impression that I thought this to be his most important contribution. With all of his exacting duties as director, chairman, professor and dean, he never lost interest in the progress of research. Not only this, he also never gave up engaging in research himself, and it was research of a high order—important, original, stimulating and carried out with a pride of workmanship that could well serve as a model for his many students. For this his memory will as long endure as for his many distinguished contributions to the organization of Science.

Ross G. Harrison

Dr. Lillie and the Founding of the Woods Hole Oceanographic Institution

The outstanding facts all have been set down by Lillie himself, in the book he wrote before he died. If you want to see the Oceanographic Institution you have only to look across the street. What I do propose is to try and tell you what kind of a man I found him during the years of our close friendship and intimate association, not about his scientific achievements.

Perhaps the characteristic which always impressed me most was his almost uncanny capability of sifting out pertinent facts in a situation, and of reaching a conclusion in a perfectly objective way, without personal bias. The result was that almost anyone else, thinking equally objectively, was almost bound to come to the same conclusion. The deliberations of the Oceanographic Institution are an excellent example. During the ten years of his presidency many questions came up regarding scientific activities, general policy, and social relations with the community where it is situated. From the very beginning he insisted that the one criterion on which decisions must be based was whether a proposed course of action would or would not likely meet the basic aims of the Institution, i.e., encourage the study of the sea. If it seemed that it would, we voted yes; if not, we voted no. And I can't remember a split vote by the Trustees during all the years of his presidency.

I do not know when the idea of an Oceanographic Institution at Woods Hole first formed in his mind, though I have no doubt that this happened long before any of us heard about it. At any rate, he did not move in the matter until the time was ripe. This appeared to be the case during the late '20's when the results of the Gulf of Maine investigations had been published, when the Navy was interested, when work was actively being done on the Pacific coast, when, in a word, oceanography appeared to be stirring in the U. S. The General Education Board, then in existence, was interested in supporting the broad range of scientific activities, and I have no doubt it was thanks to Lillie that the desirability of supporting oceanography was discussed here at Woods Hole during the conferences of 1927. He, I think, saw more clearly than anyone else that recommendations of the National Academy would have much more influence than would any backing by individuals. And evidence that such was the case was to be seen in the fact that the Rockefeller Foundation did endow the Oceanographic Institution, upon the recommendation of the Academy.

The budget of the Institution is another example of his foresight. As early as 1927, he had arrived at the tentative figure of 3 million dollars as the amount that would be required to set up and run an establishment such as he visualized. And when we sat down to figure out definite costs for building, for ships and for operations, it all added up to just that amount. A word more about the ship. I had been working on boats of one sort or another for many years and felt that an oceanographic institution could not be operated to any advantage without a seagoing vessel. When I brought this up with Dr. Lillie I was somewhat discouraged, for at first he did not seem very receptive. But it soon developed that he was in full sympathy and had been from the beginning, but that he realized, better than I, that it would be difficult to put the idea over. At that time the Foundations were quite accustomed to being asked for money for buildings, for personnel, and for libraries, but not to being asked for $200,000 for an oceanographic ship. In fact, no private institution in the world possessed such a vessel at that time. He knew very well that the only way he could put the idea over was for someone who had had practical experience with oceanographic work at sea to provide him, as ammunition, not only with a thought-out statement as to why a ship was needed, but also with all details as to the type of vessel needed, its size, its cost of construction, and its cost of operation. With this in hand, he could proceed. The result was the Atlantis.

I think I have come to the end of my fifteen minutes, but want to leave with you one other thought, and that is how close it was to Frank Lillie's heart that the Oceanographic Institution should not only be a credit to science and to the National Academy, but that it should also be a good thing for Woods Hole. I think it has been, and I hope that the rest of you think so too.

H. B. Bigelow

Frank R. Lillie and the Marine Biological Laboratory

Mr. President, Ladies and Gentlemen: I suppose you are all familar with that story of Edward Everett Hale, "My Double and How He Undid Me." This double always followed other speakers with the single sentence, "So much has been said and so well said that I need say nothing more!" I am in that position, only more so, having not only one but three predecessors on this program. Those who have gone before me have told so much of what I expected to say that I think it may be well for me to cast aside my manuscript and recite as well as I can from memory many of the contacts which I have had with Dr. Lillie during all the fifty-five years that he spent at this institution. No other person associated with the Woods Hole Laboratory has ever devoted himself so continuously and for so long a period to it. He came here, as you have already heard, from the University of Toronto where he graduated with the Bachelor of Arts degree in 1891. One of his favorite teachers there was R. Ramsey Wright. It is a significant fact that Wright had been elected a member of the Corporation of the M.B.L. in 1890. Lillie graduated in 1891. The new Woods Hole Laboratory was relatively unknown, and undoubtedly young Lillie heard of it from Prof. Wright, who became a trustee of the laboratory in the same year that Lillie came here. He was classified as an investigator receiving instruction in the report of the laboratory for the year 1891. Lillie came here at the fourth session of the laboratory. It had been going only three summers before he came, and every summer from 1891 until 1946 he had part in the work that went on here. Last summer, unfortunately, he was prevented by his fatal illness from coming. That was the first time in all those fifty-five years, which covered his entire professional life, when he was absent.

When he arrived here in 1891 he at once saw Prof. Whitman, who had the custom of at once setting his students to work on a research problem, trusting that they would get their education in the course of their research. Whitman that summer was studying leeches from the fresh water ponds in the vicinity of Woods Hole. (In those days Woods Hole was spelled Woods Holl and when the Post Office Department changed the spelling to "Hole," Whitman refused to accept the new spelling, saying that he had named some of his new species "hollensis" and that established scientifically the name Holl.) Lillie went with Whitman on some of his trips to the ponds of the vicinity and they found the pond mussel, *Unio,* bearing eggs and embryos which were caried in the gills. Whitman proposed that Lillie undertake the study of the embryology of this form, and this he did with such effect that he won the praise of all in the laboratory. At that time the study of the individual cleavage cells and their development was occupying the attention of several of us, and Lillie's work added very remarkably to the importance of this work. One of the important results of his study was the proof of the adaptation of cleavage to the future morphology of the animal that developed from the egg. This work was of such note that it led to his nomination as a member of the Corporation that same year, and to his appointment to a fellowship in Morphology at Clark University, which had been established only a few years before. Whitman was Professor of Morphology there, and a good many of Whitman's students and associates at Clark were at Woods Hole in 1891. Among them were: Bristol, Bumpus, Donaldson, Mead, Jordan, MacMurrich, Watase and Wheeler. All were either members of the faculty or graduuate students at Clark. They were a remarkable group, testimony that Whitman knew how to choose men. In the autumn of 1891 Lillie went with this group back to Clark. Conditions there were not ideal. The man who had founded the institution still lived there and felt that he ought to keep his hands on it. Whitman complained of petty annoyances such as the query, "What is the need of using so much alcohol in this laboratory?"

At that time the great University of Chicago had been re-established by the gifts of John D. Rockefeller. President Harper was looking around for faculty members—leaders. The brother of President Harper was an intimate friend of Prof. Mall, the anatomist, and Mall was instructed to sound out the members of the faculty of Clark, to see if they would go to Chicago. With very great success this was done. The entire Department of Zoölogy went to Chicago and, in addition, Mall, Donaldson, Michelson, Neff and Bolza also went. Lillie, of course, went with them. He was fellow in Animal Morphology the first year at Chicago. The second year he was appointed reader in Embryology. The third year he received the Doctor's degree, his thesis being on the Embryology of the Unionidae. In the meantime he had come every summer to Woods Hole and taught in the courses, particularly in Embryology. His ability as organizer and teacher was recognized, and he became Assistant Director of the laboratory in 1900 and was elected Director in 1908.

In the early days members of the scientific staff generally served without salaries and at the end of the summer Whitman very often went around and said, "We must have some money to pay the janitors and collectors. Can you help us?" Some of us became life members of the Corporation in that way. Those who lived long have found that bargain profitable. We have been members of the Corporation and have received the BIOLOGICAL BULLETIN for 50 years or more, merely because we went without salary which might have been paid us as teachers. Income of the laboratory in those days was from student fees, the supply department, and from minor gifts. Whitman had little fear of deficits. He was more interested in ends than in means. I said to him on one occasion, "That is going to cost a mint of money." "Great God," he said, "What is money for?" Consequently, many of the Board of Trustees, consisting of conservative Bostonians, were distressed. They passed orders with regard to not running into debt, which were disregarded by the Director. This and a system which may be characterized as "absentee landlordism" led to a break with the first board of trustees. You know how in the summer of 1897 the members of the Corporation, who were working in the old wooden building, chartered some cars on the railroad and went to Boston, 80 strong, to hold a legal corporation meeting and revise the By-Laws. At that meeting reorganization of the laboratory took place. After that, meetings of the Corporation and Trustees were to be held in Woods Hole in the summer, and not in Boston in the winter. This gave the laboratory workers a wider representation than before. It became more thoroughly national. But this did not help out a bit in the matter of finances. We were poor as ever; perhaps even worse off. Many doubted the ability of men of science to manage business affairs. I remember hearing many say that American universities and colleges had found it necessary to have a board of trustees for purposes of raising funds and to leave the faculty free to deal with subjects with which they were more familiar.

In 1901, nine years after the founding of the University of Chicago, President Harper proposed that a board of Trustees, composed of men of financial standing, be formed to run the M.B.L. and to secure funds to enable it to run without such difficulties. But the scientists in charge of the laboratory were jealous of losing control and feared that this would tie up the M.B.L. with the University of Chicago. And so it was not approved.

In 1902 the Carnegie Institution of Washington was established. The President of the M.B.L. was H. F. Osborne and one of the important trustees was E. B. Wilson. They both knew officers of the Carnegie and were asked to present to them certain projects. They proposed the support of the M.B.L. and it was accepted by the officers of the Carnegie with a certain amount of enthusiasm. But they found that they could not make it their Department of Marine Biology without owning the Laboratory outright. There was conflict of opinion among the Trustees as to whether we should surrender possession. Finally, on August 12 in that year (1902), the Corporation and Trustees of the M.B.L. voted by a great majority (60 to 3) to deed the laboratory to the Carnegie Institution. Whitman was broken-hearted; Lillie, also. Whitman wrote a stirring appeal, later published with the approval of the chairman of the Carnegie executive committee, entitled, "The Impending Crisis in the History of the Marine Biological Laboratory." The Carnegie then withdrew the proposal to take possession of the laboratory and instead proposed to give $10,000 a year for three years to help the laboratory get started on a better course. In all these crises Lillie stood with Whitman—in the first revolt of 1897 and in that of 1902. And although feeling ran high and there was a good deal of personal dissension, there was never any condemnation of the action of Dr. Lillie. He was so reasonable and so unemotional, that there was no question but that people could go along with him.

After this Prof. Whitman gradually withdrew from the laboratory. He was not present here in 1904 and 1905. His house on Centre Street was burned down in the winter of 1906 and his friends rallied and bought the property, rebuilt it, and urged him to come back. He did come back in 1906 and again in 1907, but resigned in 1908, and never returned until his body was brought here for burial in 1910. Lillie had been since 1900 Assistant Director, and when Whitman retired in 1908, Lillie, of course, was the man chosen to succeed him. From that time on, the affairs of the laboratory began to improve. The improvement was multifarious; it was not merely financial. Drew became associated with Lillie as Assistant Director in 1908, and his appointment showed Lillie's good judgment in choosing men.

In the meantime Lillie had secured the active interest of his brother-in-law, Mr. Charles R. Crane, in support of the laboratory. Mr. Crane was elected President in 1904 and continued in that office for twenty years. During all that time his gifts increased to $20,000 a year, in addition to important gifts for real estate holdings. In 1912 Mr. Crane authorized construction of the Crane building which cost at that time, before the first World War, $111,000. But the needs of additional space in a substantial building, equipped for work in bio-physics and bio-chemistry, as well as for library, lectures and administration, were especially urgent after the first World War, and plans were made for another new building. Lillie was the leader in this movement, ably supported by Dr. Drew and other members of the trustees, and with the promised support of Mr. Crane. There was also special need for increased endowment, and the trustees voted to appeal to the Rockefeller Foundation and the Carnegie Corporation for one million dollars to meet these needs. Fortunately the M.B.L. and Dr. Lillie were well and favorably known to the officers of these foundations, and in 1922 the Rockefeller Foundation voted to authorize a half million dollars for these purposes on condition that another half million be secured from other sources and that Mr. Crane capitalize his annual gifts of $20,000. The Carnegie Corporation also voted to approve a gift of $100,000 for endowment, provided that a total sum of one million dollars was secured. For nearly two years the laboratory lacked $400,000 of making up its half million to secure the half million for the Rockefeller Foundation. Finally at the end of 1923 Mr. John D. Rockefeller, Jr. requested Simon Flexner to investigate the needs of the M.B.L. and to report to him. Flexner came down to see how funds in hand were used, and whether we were worthy of increased support. I remember that at the conference here, in addition to Flexner there were Lillie, Morgan, Loeb, Wilson and myself. We all presented the case eloquently and effectively, it seemed to me, except Loeb, who said, "I am against another building. If we grow and have a big institution, we will spend all our time taking care of it." Morgan said he thought the wooden buildings were all right. I was shocked and I looked across at Lillie. He also was shocked. Then I looked anxiously at Flexner and he just winked one eye at me. I then realized that he knew the critical nature of Loeb and Morgan, and I felt relieved. Flexner went back to New York and made a favorable report, and Mr. Rockefeller personally gave the remaining $400,000 to complete the million dollar fund. Mr. Crane then endowed his annual gift of $20,000 with bonds of a par value of $405,000, and in addition agreed to meet all costs of the new building above $500,000; this ultimately amounted to $221,000. The total results of this campaign were, therefore, for the new building $721,000, for endowment $905,000, or a total of $1,626,000.

In 1924 the General Education Board gave $50,000 for the purchase of books for the Library and in 1929 it gave $200,000 for endowment of the Library. The Rockefeller Foundation gave $110,000 for the five-story library extension in 1941–42, and the Carnegie Corporation $25,000 for the purchase of books and serials. Thus the total sum of nearly $300,000 for the Library was secured from these sources. In the meantime Dr. Lillie had brought to the attention of Dr. Wickliffe Rose, President of the General Education Board, the pressing needs of safe and comfortable living quarters for workers at the laboratory, and in 1926, $250,000 was appropriated for the brick dormitory and apartment house.

Dr. Lillie wrote in his summary on the "Material Growth of the Laboratory" in his last book, *The Woods Hole Marine Biological Laboratory*, "The land, buildings, library and equipment presented expenditures of about $1,980,000 up to 1941. The major and minor endowment funds amounted to about $1,125,000; the value of the interest of the Laboratory in a biological supply house and a public garage on a very conservative capitalization of income amounts to at least $280,000. The total resources are thus $3,385,000, and there is no indebtedness." In 1900, when Dr. Lillie became Assistant Director of the laboratory, its entire assets including land, buildings, library, equipment and endowment had an estimated value of $35,000. Forty-one years later, when he retired from the Presidency, those assets were one hundred times greater. Many members of the Staff, Trustees, Corporation, and many devoted friends and organizations contributed to this great growth, but the leader in gaining the confidence and support of financiers and of great foundations was Frank R. Lillie.

His important contributions to biological research and education, his leadership in the conception and development of the Woods Hole Oceanographic Institution, his services in connection with the University of Chicago and the National Academy of Sciences were major achievements in a notable career, but I think the one thing in which he took most satisfaction was his part in the development of the Marine Biological Laboratory. Here his professional life began, here his research work first received world-wide recognition, here his ability in administration and organization was first demonstrated. Here he met the research student who became his devoted wife and helpmate, here he established his family and his hospitable summer home, here his thoughts and affections were always centered, and it is most fitting that he was buried here, where so many of his old friends and associates of the Marine Biological Laboratory are buried, almost within sight of the institution which he so much loved, and which shall be his enduring monument.

E. G. Conklin

SOME OBSERVATIONS ON THE GOLGI MATERIAL IN THE LARVAL EPIDERMAL CELLS OF DROSOPHILA MELANOGASTER

W. SIANG HSU

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Introduction

In my attempt to make a general survey on the morphology and behavior of Golgi material in a number of tissues of the larvae of Drosophila melanogaster, I have encountered in the larval epidermal cells some rather interesting phenomena which form the substance of this paper. These phenomena, besides confirming the facts and conclusions reported in my previous papers dealing with Golgi material in the larval tissues of the same fly (1947, 1948), seem to point rather definitely to a hitherto unreported function of the epidermal cells of Drosophila larvae—that of internal secretion.

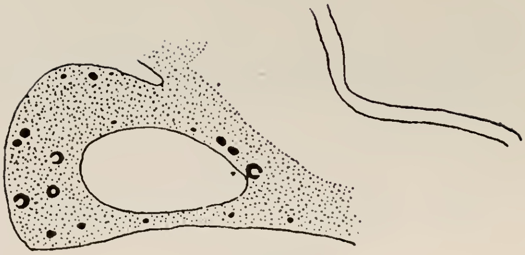
The larvae used for this study were from "wild flies." They were raised in the same cultural conditions as those used for the mid-gut study (1947). The undesirable effect of crowding the larvae was avoided by placing four or five females for only 24 hours in each bottle of 100 c.c. capacity containing approximately 10 cc. of food. Besides, only those which first reached the desired age in a fresh bottle were fixed.

For Golgi material observations, Koletchev and Mann-Kopsch techniques were employed; but the Golgi bodies in the epidermal cells, as in practically all other tissues studied in the larvae of this fly, seem to show up far better in the Mann-Kopsch slides. These slides were so much relied upon for critical observations that all figures except Figure 5 in this paper were drawn from them.

Observations and Discussion

In a section taken almost at any point along the length of a larva, most of the epidermal cells would present such appearances as shown in Figures 2 at a, 7, and 8. The cytoplasm in these cells is extremely vacuolated; and, in the vacuolated areas, apparently homogeneous bits of Golgi material of various shapes are observable. Careful focussing on these vacuolated areas, however, would usually show that they are the results of a confluence of individual small spherical globules. Evidences supporting such a view are easily obtained, once the observer is made aware of the situation. The apparent unity of many of these big and irregularly-shaped vacuolated areas would often resolve, upon careful study, into many individual small spherical globules, each still surrounded by a very thin layer of cytoplasm (Figs. 2 at b, 5 at a, 6). So, when a large irregularly-shaped vacuole is seen in a cell, it may be in a stage wherein its contributing small globules have all lost their individuality, the thin layer of cytoplasm having been withdrawn from around them (Figs. 7, 8). However, it may also be in a stage when the individual small compo-

PLATE I



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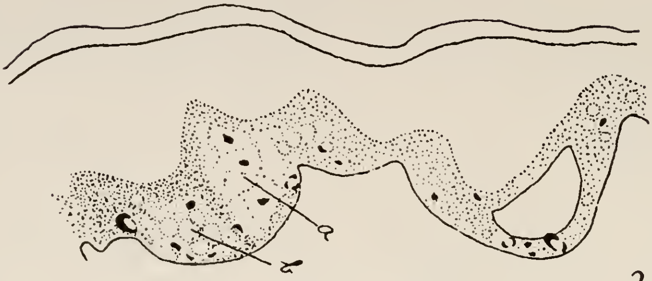

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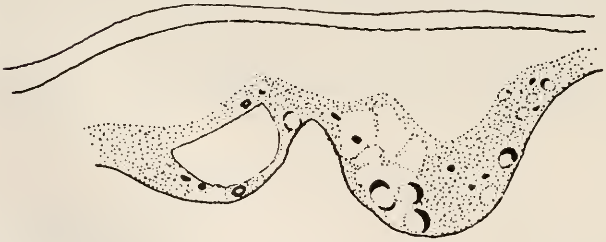

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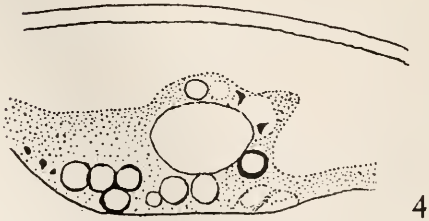

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nent globules can still be made out unmistakably (Figs. 2 at b, 5, 6). The smaller globules found within a large vacuolated area are of various sizes; and it should be noted that their sizes correspond very closely to those of the globules observed elsewhere in the cytoplasm where the formation of a large irregularly-shaped vacuole cannot as yet be easily suspected (Figs. 2, 3, 6). This is pointed out as an evidence, aside from what is indicated by the relation between the globules and the Golgi material, which will be discussed presently, that the globules both within and without the large vacuolated areas are of the same category of entities, and therefore they should have a similar origin.

Upon examining a cell at a stage of vacuolation as shown in Figures 2 at a and 7, it would be difficult to see any relation between the bits of Golgi material and the big vacuoles. But the condition as illustrated in Figure 6 reminds me rather strongly of the behavior of Golgi material in cells actively engaged in synthesizing secretion, such as have been observed in the cells of the mid-gut epithelium (1947) and those of the salivary glands of a Drosophila larva (1948). The appearance of the Golgi material in its relation to the spherical globules depicted in the same figure recalls its condition seen in the cells of both the tissues mentioned above when the individual secretion granules or droplets have grown to such a size as to be ready to be freed from the confining Golgi shell. The granular appearance of the Golgi rim around the globules is characteristic of the Golgi material when its separation product is ready to be released and itself about to break into bits of irregular shape, each of which is presumably capable of starting another cycle of secretion when proper conditions again prevail.

In addition to the observations mentioned above, my experience with the behavior of Golgi material in the glandular cells of Drosophila larvae inclined me to believe that two or three pieces of Golgi material seen in the cell represented by Figure 2 also showed familiar signs of being in the act of separating some kind of secretional material in their interior. These observations led me to a search in a large number of slides for more definite evidence in that direction. My effort was rewarded with a number of cells such as represented by Figures 3 and 4. In these

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Explanation of Plates

All the figures of the two plates are camera lucida drawings at a magnification of approximately 2200 ×.

Golgi material is represented as black dots, crescents or rings; secretion globules, as circles of broken lines; and cytoplasm, as stippled areas. A nucleus, whenever included in a figure, is indicated by an unstippled area marked out with a solid line. The epidermal cells are depicted in the figures as being over-laid with two layers of cuticula.

Plate I

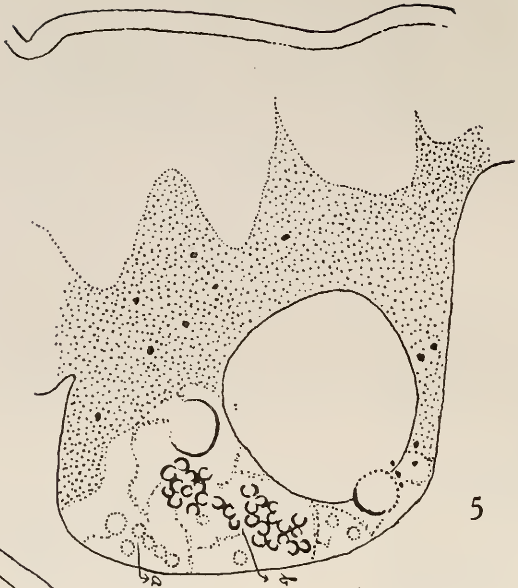
Figure 1. A cell showing in the cytoplasm some pieces of Golgi material which are apparently homogeneous and some in each of which a light center has become visible; no free secretory globules are yet observable.

Figure 2. Two cells: the one to the right showing a number of free secretory globules in the cytoplasm; the one to the left showing at b a few free globules about to be fused to produce in the cytoplasm a condition such as depicted at a.

Figure 3. Two cells showing practically all the stages of the development of a secretion globule in relation to Golgi material.

Figure 4. A cell showing the unmistakable origin of the secretory globules in the Golgi material.

PLATE II



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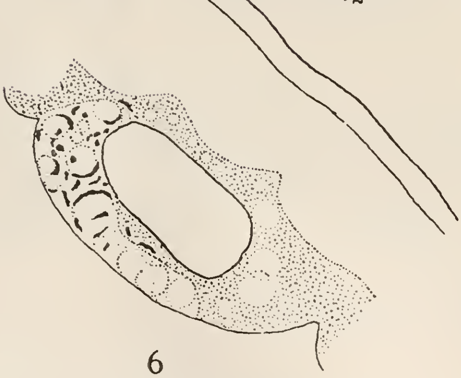

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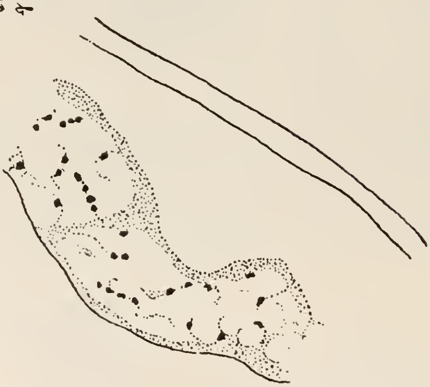

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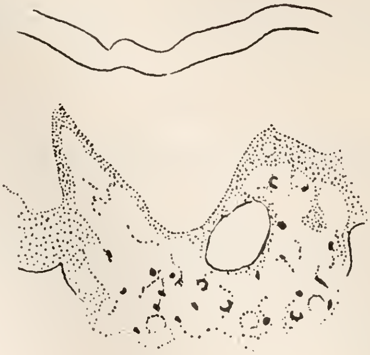

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two cells, a Golgi-material origin of the spherical globules is unmistakable. Figure 3 shows practically all the stages of the development of a secretion globule in relation to Golgi material, from apparently homogeneous bits of Golgi material to mature globules lying free in the cytoplasm.

Thus, regarding the origin, growth and releasing of the secretion globules in relation to Golgi bodies, the situation as found in the epidermal cells may be summed up as follows: Inactive pieces of Golgi material appear homogeneous; but when secretory synthesis has proceeded sufficiently far, there becomes visible one light area in each Golgi body. This area has been interpreted, on evidences which have been reported in a previous paper (1947), to be a globule of elaboration product viewed through a layer of Golgi material. As the globule increases in size, the light area in most of the Golgi bodies becomes less and less colored as a result of the continual thinning of the overlying layer of Golgi material on the surface of the globule. A time will eventually be reached when the Golgi shell will no longer be able to contain the enlarging globule within it and will mechanically break into small irregular pieces, releasing its contents into the cytoplasm. This series of changes almost exactly duplicates what has been seen in the other larval tissues of Drosophila definitely known to have a glandular function (1947, 1948).

What I consider as most instructive and significant, however, are strips of cells, usually five to six in number, which have often been observed with their cell membrane broken but with their nuclei and part of their cytoplasm still intact (Fig. 8). These cells remind one most vividly of the method of discharging their secretory product seen in the larval mid-gut epithelium and salivary gland cells of this same fly (1947, 1948). When one adds to this situation the relation observed to be existing between the Golgi bodies and the globules which they elaborate, as pointed out in the preceding paragraph, the suggestion becomes more than probable that in the Drosophila larvae, the epidermal cells may also serve as internal secretion glands. Thus, Figures 1 to 7 may be taken as showing graphically the various stages of secretory synthesis which an epidermal cell passes through—from a stage wherein the cytoplasm contains no free secretory globules but numerous Golgi bodies (some are visibly homogeneous while others show a light center) to a stage in which the cell is extremely vacuolated due to the confluence of a large number of secretory globules elaborated and set free in the cytoplasm by the Golgi bodies. Figure 8, finally, illustrates the merocrine method of discharging secretion by the cell. Having failed to see any replacement cells in the epidermis, I assume that after having

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PLATE II

FIGURE 5. A cell showing the vacuolated condition of the cytoplasm near the basement membrane. A few unfused globules are still visible within the vacuolated area at a; a group of Golgi bodies all at about the same stage of secretory synthesis are seen at b. Note the two large globules not yet freed from their respective Golgi shell and also the bits of apparently homogeneous Golgi material in the cytoplasm near the cuticula end of the cell.

FIGURE 6. A cell showing the Golgi shells around the globules already or about to be broken into small pieces which have the rugged surface and irregular shapes characteristic of apparently homogeneous and inactive bits of Golgi material.

FIGURE 7. A cell showing its cytoplasm extremely vacuolated with apparently homogeneous bits of Golgi material embedded on strands of cytoplasm; at the right end of the cell may yet be seen some globules in the process of fusing.

FIGURE 8. A cell with its cell membrane broken discharging its secretion and also a portion of its Golgi material into the body cavity of the larva.

discharged its store of secretion, each cell is capable of repairing itself and starting another cycle of secretion when proper conditions are again present. This is the situation which prevails in both the mid-gut epithelium and the salivary gland cells.

It is interesting to record that it was after I had reached the conclusion that the cells of Drosophila larvae may serve as internal secretion glands that my search into the literature for some supporting opinion led me to a paragraph by Wigglesworth (1934) in his study on ecdysis in Rhodnius, which I quote: "The histological evidence therefore favours the idea that the corpus allatum is responsible for secreting the moulting hormones which must be derived from the growing cells themselves, and this raises the question whether the general epidermal cells may not be responsible for the initial moulting hormone. This possibility cannot be entirely excluded; but the epidermal cells are not innervated, and it is therefore probable that any hormones they secrete appear only when their own growth has been stimulated by the hormone from the head." This is the first reference to the epidermal cells as internal secretion organs which has come to my knowledge, although it must be admitted that my search in the literature in that regard is not an exhaustive one. However, in quoting Wigglesworth, I do not claim that my observations prove that the epidermal cells are "responsible for the initial moulting hormone" in Drosophila larvae. Needless to say, this question merits more particular examination. I only claim that according to my material it is difficult to dismiss the idea that the epidermal cells of a Drosophila larva are capable of secreting some substance and that the secretion is discharged directly into the body cavity of the larva.

Summary

1. On the strength of observations set forth in the following paragraphs, it has been concluded that the epidermal cells of Drosophila larvae seem to act as internal secretion organs, at least at the age when the larvae are about one day before pupation.

2. The relation of the Golgi bodies to the globules, both inside and outside of the Golgi bodies as observed in the epidermal cells, has been found to be the same as what has been established in cells definitely known to be of a glandular nature in the larvae of this fly. In each piece of Golgi material, a single droplet is seen to make its first appearance and gradually to increase in size, eventually breaking free from the confining Golgi shell. It seems to be the normal procedure for the free separate secretory droplets to coalesce to form big vacuoles; and their further confluence gives to the cells in advanced secretory synthesis an extremely vacuolated appearance.

3. Many epidermal cells have been found with their cell membrane broken, thus releasing their secretion product into the body cavity of the larva. The apparent healthy condition of the nuclei of such cells and the absence of replacement cells in the epidermis would point to a merocrine mechanism of secretion in this case.

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THE ROLE OF THE SINUS GLANDS IN RETINAL PIGMENT MIGRATION IN GRAPSOID CRABS

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INTRODUCTION

Among the several functions ascribed to a hormone or hormones produced by the sinus gland of the crustacean eyestalk is that of controlling the migration of the retinal pigments. Evidence for this function has been presented chiefly by Bennitt (1932a, b), Kleinholz (1934, 1936), and Welsh (1939, 1941) and is well summarized in the reviews of Kleinholz (1942), Brown (1944), and Panouse (1947). Briefly stated, the compound eyes of decapod crustaceans typically possess three sets of pigment cells: distal melanophores forming a sleeve about each ommatidium, proximal melanin contained within the retinular cells (photoreceptive cells, proximal pigment cells), and reflecting pigment cells located among and beneath the retinular cells. In day-adaptation the proximal and distal melanins approach each other so as to surround the sensitive rhabdomes, while the reflecting pigment may be shifted proximally beneath the basement membrane of the retina. In night adaptation the proximal and distal melanins move apart in such a way as to leave the rhabdomes exposed to light from all sides, while the reflecting pigment is exposed to form a reflecting layer at the bases of the rhabdomes, visible as a reddish area of "glow" in the dark-adapted eye. The relative extent of movement of the three types of pigment differs in detail in various species of crustaceans. Attempts to demonstrate nervous control of retinal pigments in crustaceans have been unsuccessful, while the possibility that these cells are independent effectors is not supported by the available evidence. In particular, the marked diurnal movements of retinal pigments exhibited in the eyes of many crustaceans under conditions of constant darkness or of constant illumination would seem to rule out independent response to illumination as anything but an incidental factor in the normal process of pigment migration. Positive evidence for a hormonal control is based upon the discovery by Kleinholz (1934, 1936) that extracts of light-adapted *Palaemonetes* eyestalks injected into dark-adapted animals in darkness caused the distal and the reflecting pigments to move to the light-adapted position. This work was confirmed on *Cambarus* by Welsh (1939), who found that the proximal pigment was likewise induced to migrate to the light-adapted position if sufficiently strong injections of eyestalk extract were used. It has generally been assumed that the sinus gland is the source of the retinal pigment activator, and Welsh (1941) has shown that aqueous extracts of isolated sinus glands are capable of causing typical pigment migration when injected into dark-adapted crayfish. As will be reported below, similar results may be obtained in grapsoid crabs.

While the evidence for a hormonal control of crustacean retinal pigments is generally convincing, this concept has been based upon procedures which have not included one of the classical tests of endocrine function, namely, the removal of a suspected organ to produce characteristic symptoms, followed by the injection of extracts or the implantation of the organ to restore the normal conditions. Furthermore, most experimental work has been carried out with macrurans. Accordingly, it was felt that a further study of brachyurans would be of value, and that sinus gland extirpation should be made the main line of approach to the problem.

If the sinus gland is the source of retinal pigment activating hormone, its removal would be expected to stop diurnal pigment migrations and to maintain the eye in a dark-adapted state. Continued display of retinal pigment migration after sinus gland removal would, on the other hand, indicate that this organ is not the controlling factor or at least not an indispensable link in the process. Welsh (1941) has given evidence that the control of diurnal changes in retinal pigments involves central nervous activity, possibly affecting the release of hormone by the sinus gland, but the possibility that this effect can be mediated in the absence of the glands has not been investigated. The present paper reports attempts to clarify the role of the sinus glands in retinal pigment migration by means of operative sinus gland removal.

Material and Methods

A. *Animals*

Three species of grapsoid crabs common in the San Francisco Bay area have been employed: *Hemigrapsus oregonensis, H. nudus,* and *Pachygrapsus crassipes.* These animals are hardy, possess well-defined sinus glands, and show a marked "glow" in the eyes in darkness at night. With the exception of the work of Bowman (M.A. Thesis, U. C., Berkeley, Calif., 1948) no previous study of the pigmentary changes and endocrine functions of this group of crabs has been made. In order to reduce possible complications associated with egg-bearing, chiefly males have been used in the work, although enough females have been observed to indicate that their pigmentary responses are the same as displayed by the males. Animals were selected for size, since those less than 17 mm. in carapace width were too small for convenient sinus gland removal, while those exceeding 30 mm. in width were not only too large and active for easy handling under the dissecting microscope, but also possessed such thick and pigmented exoskeletons as to render difficult the observations on body chromatophores sometimes carried on concurrently with studies of retinal pigments.

All animals were kept in shallow water in individual covered glass dishes, were fed fresh liver, clam, or crab meat and had the water changed every 3 or 4 days.

B. *The preparation of active extracts*

Sinus glands and other organs to be tested for retinal pigment activating effect were taken from animals of approximately the same size as the recipients, so that dosage could be estimated in terms of a fraction of the organ in question. Recipient crabs were measured or weighed before an experiment, and a group of donor animals selected so as to have a slightly greater average width or weight than the recipients. Eyestalks were removed, split open in crab perfusion fluid, and the chain of optic ganglia with the attached sinus gland teased free with fine forceps. The sinus gland was detached and removed to a drop of distilled water in a covered one-inch Syracuse dish. The medulla terminalis was similarly placed in a second dish, and the group of three more distal ganglia (medulla interna, externa, and lamina ganglionaris) in a third dish. Other tissues extracted included brain, which in these crabs has about twice the mass of the medulla terminalis, thoracic ganglionic mass, and claw muscle. The respective tissues, when a sufficient amount had been collected, were ground with rounded glass rods, although the small sinus glands ordinarily escaped thorough crushing. Crab perfusion fluid was added to the dishes with a 1 cc. hypodermic syringe, stirred, and then taken back into a marked syringe for transfer to a small test tube. The volume of fluid used being known, a further quantity of perfusion fluid sufficient to dilute the suspension to the desired degree was added to the Syracuse dish, and likewise transferred with the marked syringe to the test tube. Ordinarily, 10 or 20 sinus glands, medullae terminalis, and distal ganglia were used, and made up to give extracts containing the equivalent of 10 organs in one cc. Brains were diluted to 5 per cc., while thoracic nerve mass and claw muscle were dissected out in quantity judged to be a little greater than the mass of brain tissue used. After transfer to test tubes, all extracts were heated in boiling water for 10 minutes, cooled, and used unfiltered. Injections were made at the base of a walking leg, the quantity injected being 0.05 cc., using for each extract the same syringe used in handling that material in preparation. Extracts stored in a refrigerator retained their potency for several days.

C. *Determination of the relative size of extracted organs*

In order to arrive at a dilution factor, so that extracts could be diluted to contain a bulk of tissue comparable to that present in an extract of sinus glands, there were dissected out in turn from each eyestalk of four crabs the sinus gland, the medulla terminalis, and the group of three distal optic ganglia. These organs from each eye were placed on a haemocytometer slide in crab perfusion fluid and compressed under the cover glass (0.1 mm. clearance). Camera lucida tracings on good quality graph paper were made, the outlined areas being cut out and weighed in groups. A test made by cutting out squares of known area showed that the combined errors in cutting and in thickness of the paper did not exceed 1.5 per cent, whereas variations in dissection were large. In Table I the relative weights of the separately dissected organs are tabulated, showing the wide variation that may be expected in removing

TABLE I

Relative sizes of sinus gland and optic ganglia

Animals		Sinus gland		Med. terminalis		3 distal ganglia	
Width	Sex	Right	Left	Right	Left	Right	Left
16.5 mm.	♀	93	97.5	1020	1070	1120	1610
17 mm.	♀	109	51	1320	1290	1690	1630
17 mm.	♂	34	26.5	1790	1300	1980	1700
19 mm.	♂	75	79	1650	1480	1650	lost
Total weights		565		10,920		11,380×8/7=13,006	
Ratio		1		19.3		23	

these small, soft organs, and the necessity of using sufficient numbers of organs in an extract to ensure even approximate consistency in results.

There are two sources of error which contribute to making the relative size of the sinus gland appear larger than is actually the case:

(1) While the ganglia could be picked free of adherent tissue, some extra tissue generally was included with the tiny sinus glands.
(2) The small size of the sinus gland allowed it to rest in the 0.1 mm. space between haemocytometer slide and cover glass without appreciable flattening. The projection of its outline thus encompassed some tissue actually less than 0.1 mm. in thickness.

These sources of error may make the sinus gland appear perhaps nearly twice its true size, relative to the optic ganglia. For purposes of dilution, however, it was assumed to have a bulk of $\frac{1}{20}$ that of the medulla terminalis and of the group of distal ganglia.

D. *Estimation of degree of dark-adaptation*

This was done by noting the extent of "glow," or light-reflecting area in the eye. Animals to be examined were picked up in dim red light and their eyes examined with the aid of a 6-volt "Mignon" lamp held close to the observer's line of vision, an operation requiring only a few seconds. Because of the irregular shape of the crab "cornea," it is difficult to make a quantitative estimate of the amount of "glow" present, especially since the "glow" is more pronounced and more often seen in the most ventral portion of the retina than elsewhere. The following scheme of expressing the degree of dark-adaptation in retinal pigments was followed, with the realization that the four stages and one doubtful intermediate stage (±) indicated are not necessarily proportional to the activity of the photo-adaptive mechanism:

Symbol	Description
0	Fully light-adapted. No glow visible.
±	Indistinct glow in ventral region of one or both eyes.
+	Glow distinct, but restricted to ventral region of eye.
++	Glow distinct in ventral region, with sub-maximal glow visible in long axis of eyestalk.
+++	Fully dark-adapted. Pronounced glow visible in long axis of eyestalk.

The use of "+" symbols was convenient in recording retinal pigment positions in those cases in which the state of body chromatophores (indicated by numbers 1–5) was studied in the same animals. The diurnal pattern of retinal pigment migrations was plotted with respect to time, the resulting curve serving to show the general trend of events. Since only three or four observations were made per day in order to avoid excessive stimulation by handling and bright light, the curves (whose exact form is of little importance in the present study) are a somewhat stylized expression of the day-to-day pattern of retinal pigment migration.

E. *Sinus gland extirpation and control operations*

Experimental animals were operated upon by means of a small dental excavating burr mounted in a jeweler's screwdriver handle, permitting the drill to be rotated with the fingers of one hand. With the crab securely wrapped in wet cloth and held under the medium power of a dissecting microscope, a hole somewhat less than 1 mm. in diameter was drilled through the dorsal side of the eyestalk. The pigmented hypodermis was then slit and laid aside with a needle, revealing (if the hole were correctly located) the sinus gland, easily recognized by its glistening bluish-white appearance against the gray of the optic ganglia. The upwelling of blood usually raised the gland so that it could be teased free with a needle or sharp-pointed watchmaker's forceps and removed. In our experience the removal of sinus glands from these crabs is easier than from the crayfish, because of the superficial location and more compact form of the gland. The wound was cauterized superficially to coagulate the blood across the opening to reduce bleeding. It was found best to wait several hours after the first operation before removing the second sinus gland. In about 10 per cent of cases the gland was broken in removal, necessitating considerable probing to retrieve all portions of it, and causing much bleeding. In cases where the opening was cut at a point not over the gland, it proved best not to attempt to seek out the gland, but to use the operation as a control. In control operations the ganglia proximal to the sinus gland were in some cases slashed with a needle point in the effort to sever the nerve supplying the gland without cutting the optic tracts; in other cases the entire optic tract was severed. The extent of injury inflicted in sinus gland removal on small crabs proved to be difficult to evaluate but was less than we have experienced in carrying out sinus gland removal in crayfish following the method of Brown (1942). The sinus gland could generally be picked from its superficial position with no apparent damage to nerves and good healing took place, yet serial sections generally revealed some degree of nerve injury, often severe enough to make it impossible to attribute the observed results simply to the absence of the sinus gland. Eyestalks were routinely fixed and sectioned serially after observations, but because of the number and the refractoriness of the heavy-walled crab eyestalks, the results of sectioning left much to be desired.

Mortality from operating was not heavy, except in the case of animals still soft from a recent moult, or those which moulted within a few days after an operation. Among animals not so weakened, deaths from bilateral sinus gland removal have amounted to 11 per cent and from operations involving nerve sectioning to 19 per cent, in some 35 and 25 animals respectively, operated upon in the series of experiments reported here. The writer recognizes that the method of sinus gland removal employed upon these small crabs does not permit the control of bleeding and minimization of nerve injury attainable with the more precise methods which Kleinholz (1947) has used upon crayfish and lobsters. It was felt, however, that results obtained on large numbers of small, responsive, easily kept animals would aid in the definition of problems to be attacked later in larger crabs kept under better conditions than have been available locally.

OBSERVATIONS

A. *The normal rhythm of retinal pigment migration*

Normal crabs kept in the laboratory showed the characteristic changes in the position of the retinal pigments in response to the daily alternation of light and darkness, exhibiting a well-marked glowing "pseudopupil" in the eyes at night and the absence of such during daylight hours. This migration stopped under continuous illumination (tested in a windowless room illuminated by two 150-watt bulbs in globes 7 feet above the animals), and the crabs remained in a continuously light-adapted state, not showing the diurnal rhythm of retinal pigment activity reported for certain other crustaceans. On the other hand, crabs kept in continuous darkness exhibited the typical diurnal rhythm of retinal pigment migration, becoming fully dark-adapted during the night hours and fully light-adapted during the day.

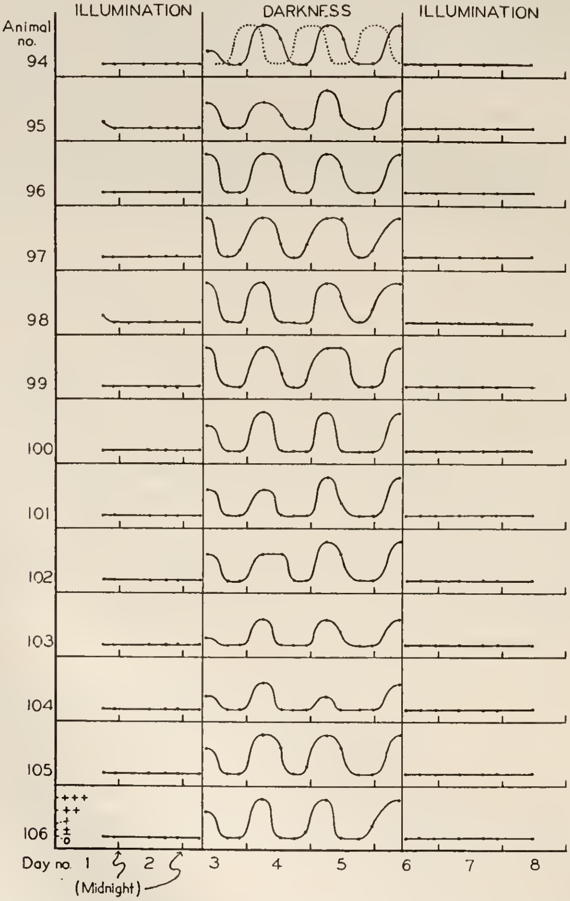

Figure 1. Diurnal migrations of retinal pigments exhibited in darkness but absent under illumination. Rhythm in darkness put out of phase with solar day by starting dark-adaptation at dawn. Dotted curve represents a "normal" rhythm.

This activity continued undiminished for at least 10 days, although occasional animals gradually became "out of phase" with the solar day. Ordinarily night-adaptation commenced in the late afternoon, even before sunset, while the reverse process of day-adaptation was often under way before dawn. The most striking case of a shift in phase of the diurnal activity cycle occurred when a group of *Hemigrapsus oregonensis* which had been exposed to continuous light for 40 hours were transferred to the darkroom at 7 A.M. They soon became dark-adapted, and thereafter exhibited a period of dark-adaptation starting after midnight and lasting until early afternoon—an example of the reality of a diurnal activity rhythm as well as of its lability (Fig. 1).

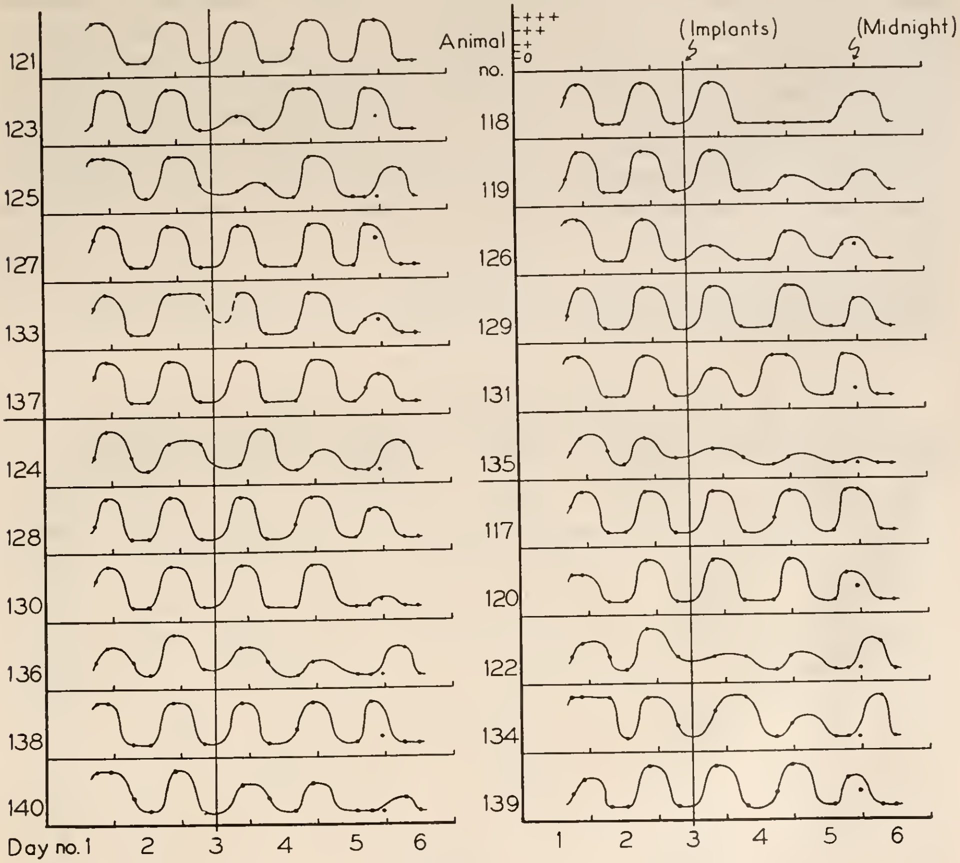

FIGURE 2. Relative ineffectiveness of a sinus gland or other tissue (implanted on third day) in suppressing diurnal rhythm of retinal pigments of crabs kept in darkness. 121–137, one sinus gland; 124–140, one medulla terminalis; 118–135, one group of three distal optic ganglia; 117–139, muscle. Note that on the fifth night too close spacing (95 minutes) between observations decreased the degree of dark-adaptation obtained.

B. *The effects of injections of sinus gland and nervous-tissue extracts*

Over 40 tests were made in which sinus glands or optic ganglia were implanted by the technique of Scudamore (1947, p. 190) into the bases of walking legs of crabs the same size as the donors. The recipient crabs were observed in constant darkness to determine if the implanted tissues would suppress the normal diurnal cycle of the retinal pigments. While there was some evidence that a factor active upon retinal pigments was present in nervous tissues as well as in sinus glands, the results were not conclusive, as shown in Figure 2, which is typical of the whole series. On the other hand, extracts of whole eyestalks injected into night-adapted animals of all three species in darkness caused a migration of retinal pigments to the "day" position, as evidenced by a complete or nearly complete disappearance of the "glow." Since it had early been observed that if crabs were inspected for "glow" in the darkroom at night, there often occurred a transitory change to a partially light-adapted state within the hour or two after the illumination of the eyes (as shown in Fig. 2 fifth night), and since these crabs only became dark-adapted during the night hours it was necessary to time injections in such a way that all animals could be safely assumed to be night-adapted at the time of injection without making an inspection. The procedure adopted was as follows: a group of 24 crabs were measured, placed in numbered dishes, and brought into the darkroom at about 6 P.M. Inspection at a later hour that evening and again during the following day sufficed to show that the animals were exhibiting the normal diurnal rhythm. They were then presumed to be night-adapted by 9 or 10 P.M. of the second evening. Injections were made under dim red light, the time of each being noted. At one hour after injection, each animal was inspected for "glow." This procedure was repeated the next two eve-

TABLE II

Effects of sinus gland and optic ganglia extracts, tested upon Hemigrapsus oregonensis

Crab no.	Width (mm.)	March 7		March 8		March 9	
		Injection	Result	Injection	Result	Injection	Result
1	21	Control injection	+++	½ sinus gland	+	Muscle	+++
2	19		++		0		++
3	17		+++		±		+++
4	19		+++		+		+++
5	20	½ medulla terminalis	+	Control injection	+++	½ sinus gland	Died
6	18		+		+++		0
7	17		+		+++		+
8	20		+		+++		±
9	19	1/40 medulla terminalis	++	½ medulla terminalis	+	Control injection	+++
10	17		++		+		+++
11	21		++		+		+++
12	18		++		+		+
13	20	½ distal ganglia	+	1/40 distal ganglia	+++	½ medulla terminalis	±
14	19		+		++		±
15	16		±		+++		±
16	18		+		+++		+
17	21	Muscle	+++	½ distal ganglia	+	1/40 sinus gland	+
18	18		+++		+		++
19	17		+++		±		+
20	18		+++		+		++
21	17	½ sinus gland	+	Muscle	+++	½ distal ganglia	0
22	18		+		+++		+
23	17		±		++		±
24	17		0		+++		0

nings, so that injections were given to the same group of crabs for three successive nights. Since the injections often interrupted the normal pattern of diurnal migration of pigments, it was surprising that consistent results could be obtained even for three nights. After such a series of injections, the test animals were discarded, since the tendency to get out of phase with the solar day introduced an element of uncertainty.

There appear, in Tables II and III, the results of two such series of injections of extracts of sinus gland, nervous, and muscular tissues. Extracts were from light-adapted crabs of the same species and of the same average size as the recipients, and all injections were of 0.05 cc. of extract, the concentrations being expressed as the fraction of an "average" organ injected. A given extract was used for one series of three successive injections. Control injections were of crab perfusion fluid.

TABLE III

Effects of extracts of brain, thoracic nerve mass, optic ganglia and sinus gland, tested upon Hemigrapsus oregonensis

Crab no.	Width (mm.)	March 14		March 15		March 16	
		Injection	Result	Injection	Result	Injection	Result
25	18	Brain ½ hemisphere	+	Brain 1/40 hemisphere	++	1/10 thoracic nerve mass	+
26	20		++		+++		±
27	19		+		+		Died
28	21		+		++		+
29	17	1/10 thoracic nerve mass	++	1/200 thoracic nerve mass	++	½ medulla terminalis	±
30	18		++		+++		±
31	17		++		++		±
32	20		++		++		+
33	18	½ medulla terminalis	±	1/40 medulla terminalis	++	Muscle	+++
34	18		±		++		++
35	18		+		++		+++
36	19		++		++		+++
37	18	Muscle	+++	Muscle	+++	½ sinus gland	0
38	18		+++		+++		±
39	18		+++		+++		0
40	21		+++		+++		±
41	17	½ sinus gland	+	1/40 sinus gland	+	Control injection	+++
42	21		+		+		+++
43	16		±		+		+++
44	17		±		++		++
45	17	Control injection	+++	Control injection	+++	Brain ½ hemisphere	+
46	20		+++		++		±
47	17		+++		+++		Died
48	20		+++		+++		+

While these injection methods are only roughly quantitative, several generalizations may be drawn from the results:

(1) The total retinal pigment activating potency of one sinus gland is roughly equivalent to that found in one medulla terminalis, or in one group of the three more distal optic ganglia.

(2) Since the bulk of either of these ganglionic masses is at least 20 times that of the sinus gland, it would appear that the active principle is at least 20 times more concentrated in the latter organ.

(3) Of the total active substance extractable from the eyestalk, roughly ⅓ resides in the sinus gland. This is in contrast to the distribution of body-chromatophore-activator in the eyestalks of several species of shrimps and crabs studied by Brown (1940) in which at least 80 per cent of the total activity resides in the sinus gland.

(4) Nervous tissue other than optic ganglia also contains a retinal pigment activating principle, in a concentration of the same order of magnitude as is found in optic ganglia.

The above facts suggest that the production of retinal pigment activator is not restricted to the sinus gland, but occurs in other parts, or perhaps all parts, of the nervous system. The active substance may, however, be more efficiently produced or quantitatively stored in the sinus gland, which in turn might be specialized for its periodic release. Upon this basis, attempts were made to block the diurnal retinal pigment changes by operative removal of sinus glands.

C. *Sinus gland removal in animals exposed to normal darkness and daylight*

Bilateral sinus gland removal was carried out upon a group of 13 crabs and the animals observed daily and nightly for periods of 3–5 days. The presence or absence of "glow" was noted but sufficient data to show the time course of pigment migration were not collected. The results of sinus gland removal, as observed in the living animals, were quite variable, but in no case did a glow appear during daylight as the result of the operation. A glow was generally present at night, but was variable in amount, ranging from a practically normal glow down to a slight or questionable glow. Both eyes of each of these animals were fixed and sectioned serially, one eye being removed in bright daylight, and the other at night, under dim red light. A summary of the observations with the results of a study of serial sections of the eyestalks follows (Table IV).

As a control operation, the optic tract was severed within the eyestalks of 8 animals by the severe procedure of stabbing the region of the medulla terminalis or interna with a heated needle, after drilling a hole as for sinus gland removal. The sinus gland itself was not touched. With two exceptions the animals in this group failed to show night adaptation. Histological examination revealed that in at least three animals, possibly because of damage to blood supply, the retina and distal optic ganglia were in a necrotic state. In such animals the apparent state of light-adaptation is perhaps comparable to that reported by Bennitt (1924) as due to death or to separation of the eyestalk from the body rather than to processes related to denervation or interference with sinus gland function. The results obtained on the remaining five animals are summarized in Table V.

The effect of optic nerve severance seems clearly to be a continued state of day-adaptation, a result consistent with the view advanced by Welsh (1941) that the light-adapted state is brought about by the release of a sinus gland hormone when a tonic nervous inhibition of the gland is reduced. Yet the view that a sinus gland

TABLE IV

Results of histological examination; sinus gland removal; normal daylight and darkness

Record number	Species	Response	Sinus glands	Nerve damage
9	*H. oregonensis*	Normal	Complete removal	Slight
11	*H. oregonensis*	Right: normal Left: impaired night adaptation	Complete removal	Right: slight Left: considerable
13	*H. oregonensis*	Impaired night condition	Complete removal	Right: optic tract severed Left: some damage
16	*H. oregonensis*	Right: intermediate Left: normal	Complete removal	Right: necrotic Left: slight damage
18	*P. crassipes*	Normal	Complete removal	Slight
21	*P. crassipes*	Impaired night adaptation	Right: removed Left: some remaining	Right: slight Left: severe
24	*H. nudus*	Normal	Complete removal	Slight
39	*H. nudus*	Impaired night adaptation	Complete removal	Right: severe Left: considerable
40	*H. nudus*	Normal	Complete removal	Slight
46	*H. nudus*	Impaired night adaptation	Complete removal	Moderate to severe
47	*H. nudus*	Right: normal Left: impaired night adaptation	Right: some remaining Left: removed	Right: slight Left: optic tract severed (?)
49	*H. nudus*	Impaired night adaptation	Right: trace remaining Left: removed	Slight
50	*H. nudus*	Impaired night adaptation	Right: trace remaining Left: removed	Slight

hormone is solely responsible for inducing the movement of pigment to the "day" position is not supported by the results of sinus gland removal, which in no case caused the eye to become permanently "dark-adapted;" in fact any changes that were produced were in the direction of increased day-adaptation, a result possibly attributable to nerve damage incidental to sinus gland removal. Furthermore, the possibility exists that, in the absence of sinus gland hormone, the retinal pigments might possess some power to act as independent effectors in a direct response to light. Accordingly other series of observations were made under conditions of continuous darkness.

TABLE V

Results of histological examination; optic nerve sectioning; normal daylight and darkness

Record number	Species	Response	Sinus glands	Nerve damage
12	*H. oregonensis*	Permanent "day" adaptation	Damaged (?)	Optic tracts severed
25	*P. crassipes*	Permanent "day" adaptation	Intact	Optic tracts severed
41	*H. nudus*	Permanent "day" adaptation	Right: intact Left: damaged	Right: optic tract severely damaged Left: optic tract severed
42	*H. nudus*	Right: day adapted Left: nearly normal	Intact	Right: optic tract severed Left: optic tract severely damaged
45	*H. nudus*	Right: day adapted Left: impaired night adaptation	Intact	Right: optic tract severed Left: optic tract damaged

D. *Sinus gland removal in animals exposed to continuous darkness*

The effects of sinus gland removal and optic tract damage were tested upon a group of 25 animals of all three species, both males and females, selected to include no soft, recently moulted crabs. In the course of the experiment, five moulted and did not survive. Observations were first made on the intact animals for three days and nights, to obtain a record of the pattern of the diurnal rhythm in each animal (see Fig. 3). On the fourth day the group was returned to a lighted room, fed, given fresh seawater, and received the first operation upon the right eyestalk. On the following day the second operation was performed on the left eyestalk, and the animals returned to darkness. Observations continued over the next five days and nights (with one night on which no check could be made). By reference to Figure 3 it may be seen that in the four normal control animals the rhythm continued unchanged.

The results of operations again showed considerable variation. In this series control operations were restricted to a superficial cut across the optic tracts proximal to the sinus glands, in the effort to denervate the gland without severing the main optic tracts. Of the six survivors, four showed no real change, while in two animals (nos. 57 and 63) one eye continued to show diurnal changes and the other did not. Histological examination of the eyes in this group seemed to indicate that in those two eyes which failed to show pigment migration, nerve damage was more severe than in those eyes which continued to show diurnal pigment migration, but the evidence was far from satisfactory. The fact that the two eyes of a single animal may show different degrees of adaptation has been noted often enough in this work to indicate a considerable degree of independent control of the pigments in each eye.

Ten animals survived bilateral sinus gland extirpation (of these, nos. 54, 65, and 76 had the left eyestalk removed, observations being carried out on the right

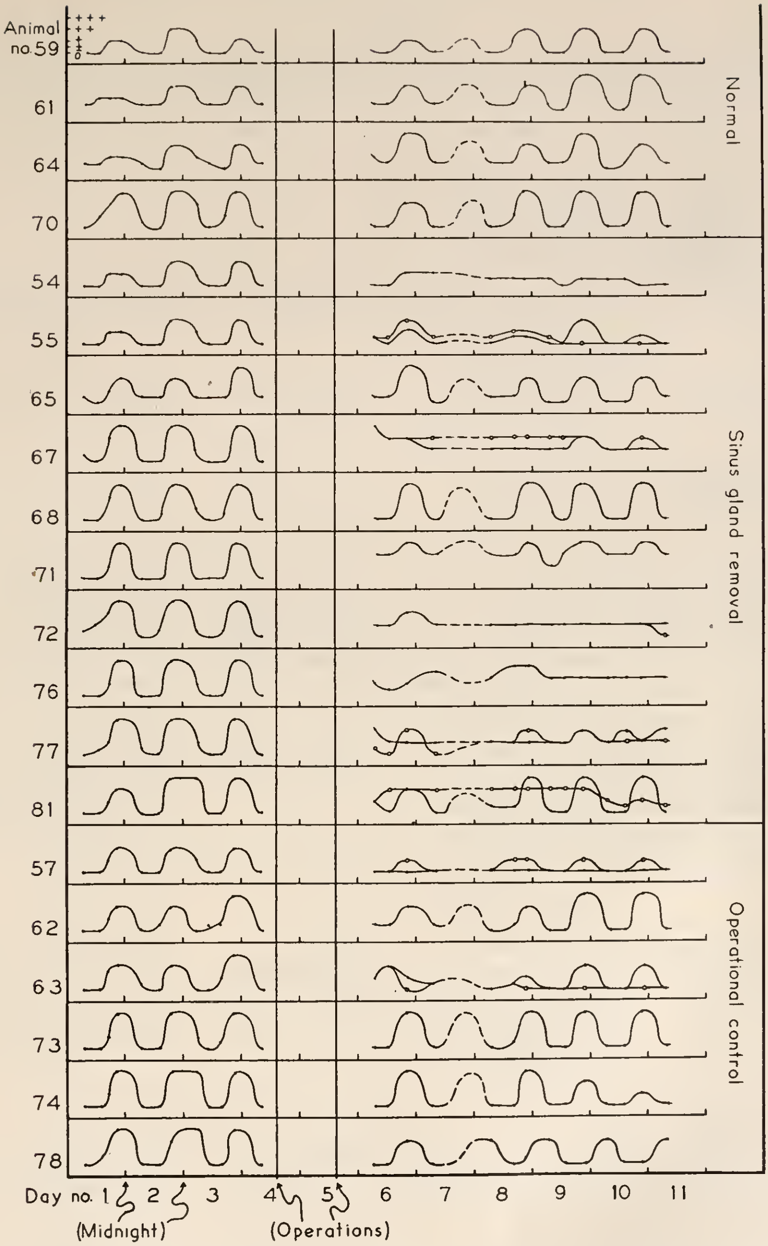

FIGURE 3. The effects of sinus gland removal and optic nerve damage upon the diurnal rhythm of retinal pigment migration of crabs kept in darkness. Double curves indicate a difference in the two eyes of one animal, the open circles indicating the left eye. Single curves indicate that both eyes were the same.

TABLE VI

Results of histological examination; sinus gland removal and control operations; continuous darkness

Record number	Species	Response	Sinus glands	Nerve damage
59	*H. nudus*	Normal (control)	—	—
61	*P. crassipes*	Normal (control)	—	—
64	*P. crassipes*	Normal (control)	—	—
70	*H. oregonensis*	Normal (control)	—	—
54	*H. nudus*	Right: approaching "day" state Left eye removed	Removed —	Optic tract severed —
55	*H. nudus*	Right: impaired "night" migrations Left: "day" condition	Right: trace Left: removed	Slight Severe
65	*P. crassipes*	Right: normal Left eye removed	Right: trace —	Slight —
67	*H. oregonensis*	Both: intermediate	Both: removed	Both: moderate
68	*H. oregonensis*	Both: normal	Right: trace Left: removed	Both: slight
71	*H. oregonensis*	Both: migrations, tending to "night" state	Both: removed	Both: moderate
72	*H. oregonensis*	Both: intermediate	Both: removed	Both: moderate
76	*H. oregonensis*	Right: intermediate Left eye removed	(?) —	(Lost in sectioning; no evaluation)
77	*H. oregonensis*	Both: intermediate, slight migrations	Both: removed	Right: severe Left: moderate
81	*H. oregonensis*	Right: normal Left: intermediate	Right: (?) Left: removed	Right: (lost in sectioning) Left: moderate
57	*H. nudus*	Right: "day" state Left: impaired night migrations	Both: intact	Right: optic tract severed Left: moderate
62	*P. crassipes*	Both: normal	—	(Not sectioned)
63	*P. crassipes*	Right: nearly normal Left: "day" condition	Both: intact	Right: slight Left: optic tract severed
73	*H. oregonensis*	Both: normal	Both: intact	Both: slight
74	*H. oregonensis*	Right: normal Left: (not operated upon)	Right: intact Left: (intact)	Right: slight Left: —
78	*H. oregonensis*	Both: normal	—	(Not sectioned)

eye only). The results were quite variable, nos. 54, 67, 72, and 76 exhibiting a loss of diurnal pigment movements; nos. 65, 68, and 71 continuing to show these changes; and nos. 55, 77, and 81 exhibiting diurnal rhythm in the pigments of one eye and not the other. Histological examination of the eyes in this group showed that there was great variation in the extensiveness of nerve damage in different cases. The following summary (Table VI) indicates the extent to which histological results (admittedly unsatisfactory) agree with the observed behavior.

The results shown in Figure 3 and Table VI bear out the earlier indications that severe damage to nerves can cause a persistent state of day-adaptation in the retinal pigments. The effects of sinus gland removal are less easy to evaluate. In the case of three animals (nos. 55, 65, and 68) the possibility of traces of the sinus gland remaining in the eyestalk cannot be eliminated. Even in cases where the gland itself is clearly absent, its nerve may be seen extending far into the medulla terminalis. Since the sinus gland nerve in life exhibits the same characteristic bluish appearance as the sinus gland itself, and shows in fixed material the presence of an eosinophilic secretion product, it must be admitted that it may well be capable of compensating to some extent for the loss of the gland proper. Perhaps of more significance are those animals (nos. 67, 71, 72, 76, 77, and the left eye of 81) in which sinus gland removal is followed by incomplete day-adaptation, and in which nerve damage, though present, is not excessive. Although sinus gland removal has failed, as before, to produce a full and persistent night-adapted state, it seems at least in these cases to have produced a shift in that direction. Since nerve damage tends to produce the day-adapted state, the intermediate condition may be the resultant of the effect of sinus gland removal promoting dark-adaptation and nerve damage causing light-adaptation. The fact that this "tendency" toward dark-adaptation is not wholly effective even in continuous darkness, does not in itself encourage one to assign an exclusive role in retinal pigment control to the sinus gland. Likewise the fact that the eyes of the same animal may behave differently is more easily attributable to unequal nerve damage than to sinus gland removal.

DISCUSSION

The studies reported above were undertaken in an attempt to verify the generally accepted claim that day-adaptation in the decapod crustacean eye occurs under the influence of a hormone produced and released by the sinus glands. The work upon which this view is based has been done chiefly upon macrurans: shrimps and crayfish. The critical test of sinus gland removal still remains to be applied to the macrurans. Its application in the brachyurans, reported herein, while generally supporting previous contentions regarding the macrurans, does indicate that at least in the grapsoid crabs the sinus gland is not the sole source of retinal pigment activator. Despite numerous operations, simple sinus gland removal has usually failed either to cause the eye to remain dark-adapted or to cease its diurnal pigmentary changes, except that in continuous darkness there may be a lessened ability to assume the day-adapted condition during daylight hours. In all types of operations there has been shown a marked tendency for nerve damage to result in a more pronounced state of day-adaptation, a factor which should be taken into account when describing the effects of operations upon the sinus gland. The injection of extracts of other tissues indicates that while the sinus gland is clearly richer in active material, nervous tissues contain large amounts, the sinus gland nerve showing visible secretion products like the sinus gland. If this material is utilized as a hormone, we are forced to consider the possibility that it can be released by nervous tissues in significant amounts, even in the virtual or complete absence of sinus glands.

SUMMARY

1. The grapsoid crabs, *Hemigrapsus* and *Pachygrapsus,* exhibit in constant darkness a marked diurnal rhythm of retinal pigment migration.

2. This rhythm is absent in continuous light, and can be induced to become out of phase with solar day in constant darkness.

3. About ⅓ of the retinal pigment activator of the eyestalk resides in the sinus gland, with the remaining ⅔ distributed in the optic ganglia. Brain and other nervous tissues also contain an active principle in concentrations comparable to that in optic ganglia.

4. The sinus gland is at least 20 times richer than nervous tissue in retinal pigment activator.

5. Damage to nerves of the optic tract impairs the attainment of night-adaptation. If sufficiently severe, nerve damage may result in a state of permanent day-adaptation.

6. Operative sinus gland removal does not produce full dark-adaptation, a fact which may be in part explainable on the basis of concomitant nerve damage, but it does reduce the extent of day-adaptation in crabs kept in constant darkness.

7. It is concluded that, in the grapsoid crabs studied, the sinus glands are specialized (in addition to entirely different functions) for the elaboration and possibly the release of a principle active upon retinal pigments. On the present evidence it cannot be concluded that the sinus glands are the sole source of the retinal pigment activating hormone(s) in this group of crabs.

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THE PROTHORACIC GLANDS OF LEUCOPHAEA MADERAE (ORTHOPTERA)

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Introduction

Of the insect organs which furnish developmental hormones the corpora cardiaca-allata and the neurosecretory centers of the brain have been known for some time to be operative in a number of orders. More recently the endocrine role of another organ of internal secretion, the prothoracic glands, has been demonstrated in *Lepidoptera*. In this group of insects an active principle produced by these glands is necessary for pupation and imaginal differentiation, and apparently also for molting (Fukuda, 1940, 1941; Williams, 1947). These observations suggest that the factor produced by the prothoracic glands should be classified as a growth and differentiation hormone (see Scharrer, in press).

In a recent publication Williams (1948) discussed the still meager histological data available at present in regard to the prothoracic glands of certain *Lepidoptera*. In the same paper he mentioned the existence of prothoracic glands in the larva of *Cimbex americana* (*Hymenoptera*), and suggested that the organs described as "ventral glands" in *Dixippus morosus* (*Orthoptera*) by Pflugfelder (1938, 1939) may be homologous to the prothoracic glands of the *Lepidoptera*. Aside from these data there is little morphological evidence of the occurrence of prothoracic glands in insects other than *Lepidoptera*.

Since the existing experimental evidence suggests an important role of the prothoracic glands, it appears desirable to study their occurrence among various groups of insects, their histology and appearance in different phases of the life cycle, and their relationship to other endocrine organs. The investigation reported here concerns the histology of the prothoracic glands of *Leucophaea maderae* (*Orthoptera*) in different stages of postembryonic development and their involution after metamorphosis.

Material and Methods

The observations are based on sectioned tissue (74 specimens) and on supravitally stained material (over 60 specimens). The animals used were various nymphal instars in different phases of the intermolt period, as well as male and female imagoes of *Leucophaea maderae* ranging from one hour to 15 months of "adult age." In the freshly opened insect the prothoracic glands are very difficult to see on account of their transparency which does not permit their differentiation from neighboring structures. The identification of the paired organ is greatly facilitated by the use of supravital dyes with which the prothoracic glands stain conspicuously. Specimens having received injections of neutral red or methylene blue solutions prior to dissection offer a suitable material for the study of certain morphological features of the gland. For permanent preparations the prothoracic region was fixed in Zenker-formol, Bouin, or Carothers solution. Paraffin sections 3–7 micra thick were stained with the following techniques: Hematoxylin-eosin, Mallory-azan, Masson's and Lendrum's trichrome, and Wilder's reticulum stains. Isolated glands were incubated at 56° C. in 2 per cent osmic acid solution for several hours and were studied in toto or after paraffin embedding.[2]

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[1] The work was done while the author held a Fellowship from the John Simon Guggenheim Foundation. Support by grants from the American Cancer Society and The Anna Fuller Fund is gratefully acknowledged.

OBSERVATIONS

The prothoracic glands of *Leucophaea* consist of two bands of tissue which occupy a ventral position in the anterior thorax in close proximity to the prothoracic ganglion. The two bands cross in the manner indicated in Figure 1; at the point of crossing the organs are connected by a narrow tissue bridge. The anterior ends of the tissue bands taper off in the neck region. At the posterior end each band divides into two short branches one of which establishes nervous connection with the prothoracic ganglion. Figure 2, based on methylene blue preparations, shows a branch of a thin nerve entering the prothoracic gland shortly after its emergence from the lateral surface of the ganglion. No ducts are found in connection with

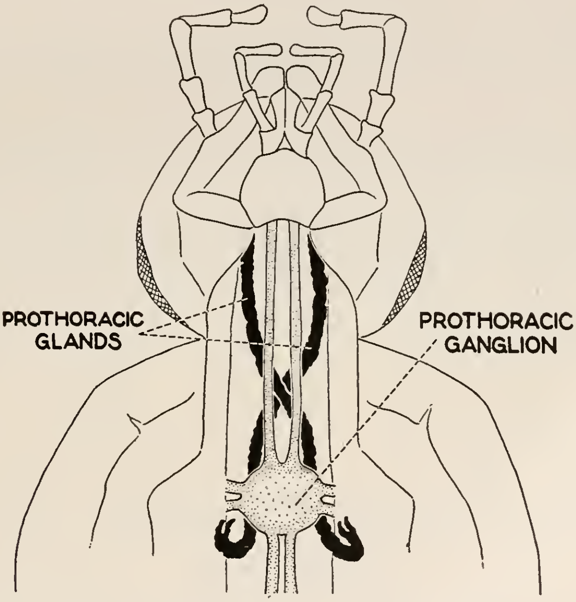

FIGURE 1. Ventral aspect of anterior thorax and head (tilted back) of *Leucophaea* nymph showing position of prothoracic glands.

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[2] I am indebted to Miss Kate Gruen for valuable technical assistance.

these glands; they are surrounded by blood spaces. It may be assumed, therefore, that the secretory products are given off into the blood.

The prothoracic glands are present in both nymphs and adults, but in the latter they become considerably reduced in size and structure soon after emergence.

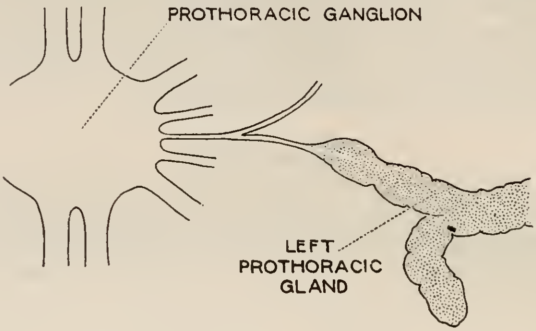

FIGURE 2. Innervation of prothoracic gland of *Leucophaea* by a lateral branch from the prothoracic ganglion, as shown for left side. Diagram based on methylene blue preparations.

A. The nymphal type

In the prothoracic gland of the nymph and of the freshly emerged adult the cells are arranged in densely packed layers around the longitudinal axis of the tissue band. The center is occupied by a trachea, a nerve, and several parallel fibers of striated muscle all of which extend throughout the length of the organ (Figs. 3, 4). The nerve is evidently derived from the prothoracic ganglion, since no other nervous branch than the one mentioned above (Fig. 2) has been observed to enter the gland. The central nerve, in addition to innervating the musculature of the organ, probably also supplies the glandular tissue; the delicate branches of the nerve could be traced only for short distances in methylene blue preparations. Due to the contraction of the axial muscle the glandular tissue in fresh and in fixed preparations is more or

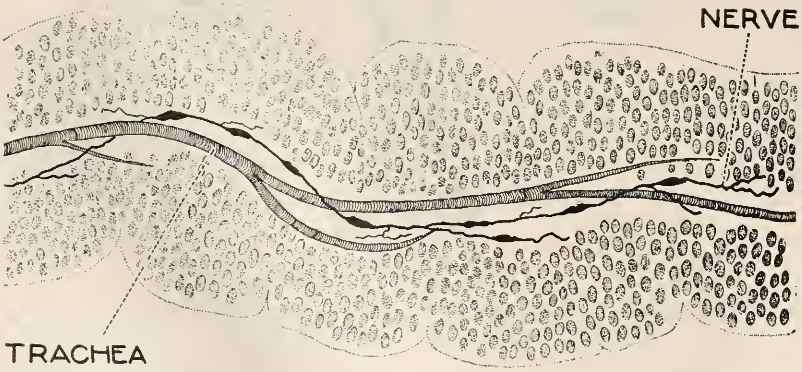

FIGURE 3. Prothoracic gland of nymph of *Leucophaea* stained supravitally with methylene blue.

less folded, with the result that the width of the organ varies. In the wider portions 8 to 12 nuclei may be counted across the width of the cellular layer of one side (Fig. 3).

The deeply staining nuclei are the most prominent feature of the gland. They are ovoid and, for the most part, approximately uniform in size and appearance. In regard to their dense arrangement and general morphology they resemble the nuclei of the corpora allata of the same species. In some nymphal glands a number of considerably larger nuclei may be observed. The majority of specimens studied showed some pycnotic nuclei in the prothoracic glands.

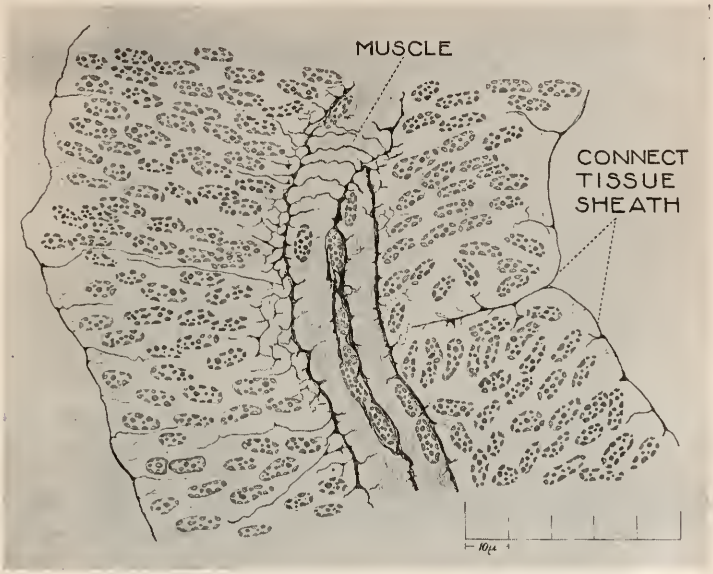

FIGURE 4. Prothoracic gland of last instar female nymph of *Leucophaea* in longitudinal section. Paraffin, 4 micra, Mallory azan.

The cytoplasm is not abundant and the cell boundaries are difficult to discern, except under favorable conditions as, for instance, in the periphery of the sectioned organ, where the nuclei are less densely packed.

With the methods employed for permanent preparations no appreciable amount of cytoplasmic inclusions indicating a secretory activity of the prothoracic gland cells was observed. In two specimens a few acidophilic granules were seen, in others the cytoplasm showed vacuolization. These observations suggest the possibility that certain cytoplasmic inclusions may be dissolved during the ordinary embedding and staining procedure. This view is further supported by the results of supravital staining. The cytoplasm of fresh tissue subjected to supravital dyes for a suitable period of time contains inclusions, as indicated in Figure 5. Round bodies varying in diameter stain a distinct blue with methylene blue. The staining is not always uniform throughout the particle; more deeply stained areas may be differentiated from a lighter background. The granular inclusions found to stain red with neutral red are the same as those taking up methylene blue. This can be demonstrated by placing a methylene blue treated organ in a drop of weak neutral red solution on a slide. When a cover slip is used, the blue stain soon disappears from the granules and is replaced by a red tint. The same individual granules can be observed as they change color.

After incubation with osmic acid the cells of the prothoracic glands show blackened inclusions which, on account of their size and distribution, seem to be identical with the granules appearing in supravitally stained organs. These observations suggest that the granular inclusions are lipid in nature. Further than that no definite conclusions can be drawn. On the one hand, these bodies may be interpreted as cytological manifestations of a secretory activity of the prothoracic glands, since it is known that certain types of secretion granules stain with neutral red. On the other hand the inclusion bodies may be identified as Golgi material on the basis of their similarity, both as to appearance and stainability, with the Golgi elements described in other invertebrate material (Worley, 1944).

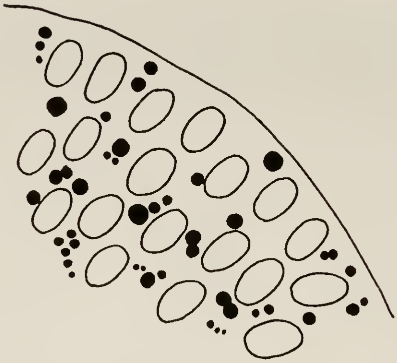

FIGURE 5. Glandular portion of prothoracic gland of last instar nymph of *Leucophaea*, supravitally stained, showing granular inclusions (solid black) and nuclei (in outline).

The prothoracic glands are ensheathed by a thin connective tissue membrane from which tenuous branches may be seen to enter the glandular tissue. These branches continue into the fibrous network surrounding the muscle elements in the center (Figs. 4, 6). These relationships are best observed in azan preparations where the fibrous elements stain a bright blue.

The cells of the prothoracic glands undergo mitotic divisions. Not all specimens studied showed mitoses. In a group of 44 dated nymphs, ranging from instar four to eight, 4 micra serial sections in the horizontal plane through the prothoracic glands were checked for mitotic figures. Specimens fixed immediately after molting, or at intervals up to six days following a molt, had no mitoses; neither did nymphs preparing for a new molt as indicated by the separation of the old cuticle from the epidermis. However, a certain number of the prothoracic glands from animals fixed during the remainder of the intermolt period, showed mitotic figures. While some of these specimens had low values (approximately one mitosis in ten sections), others had an average of up to eight mitoses per section. Since the intervals between two molts in *Leucophaea* are subject to considerable variation, no more precise conclusion can be drawn than that at some time during the intermolt period a probably short, but considerable spurt of mitotic activity takes place in the prothoracic glands.

B. Adult type

In the male and female imago of more than eight days of "adult age" the prothoracic glands are considerably changed in appearance (Fig. 7). The tissue bands have become thin and the microscopic examination shows that they consist almost exclusively of the muscular core. Most, if not all, of the glandular cells have disappeared. The connective tissue elements are more conspicuous than in the nymphal gland. This change of morphological appearance of the prothoracic glands within the short period of transition from the nymphal to the adult life may be interpreted as an indication that in the imago these organs are no longer functionally active. The steps of this striking involutionary process were traced in a series of adult male and female specimens, fixed at daily intervals after emergence. While the prothoracic glands of the freshly emerged adult resemble the nymphal organ, the first signs of regression may be noticed after 24 hours of adult life. At this stage a small number of the nuclei of the glandular component are pycnotic. Within the next three or four days the cellular breakdown becomes very conspicuous in that a large proportion of pycnotic nuclei or cellular remnants are interspersed with a gradually decreasing number of seemingly still normal nuclei (Fig. 6). The glandular tissue decreases in width, while no apparent change takes place with re-

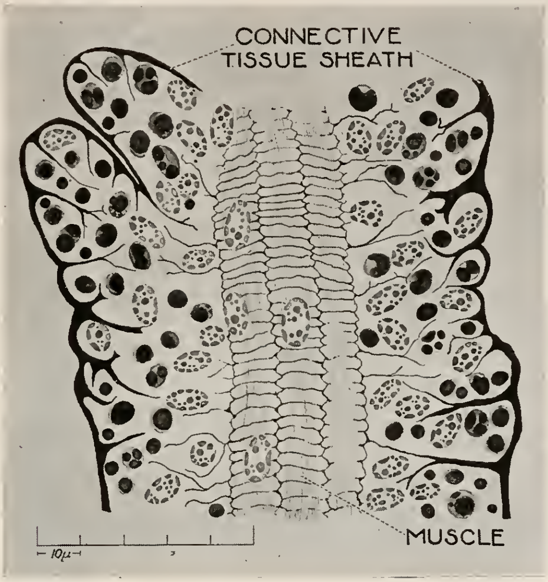

FIGURE 6. Longitudinal section of prothoracic gland of male adult *Leucophaea* four days after emergence, showing involutionary process (nuclear breakdown, decrease in width of glandular component). Paraffin, 4 micra, Mallory azan.

spect to the muscular component. Animals fixed six, seven, or eight days after emergence show the involution of the prothoracic glands almost completed, practically all of the glandular tissue having disappeared by this time. From this stage on throughout adult life the organs show the same picture of involution (Fig. 7).

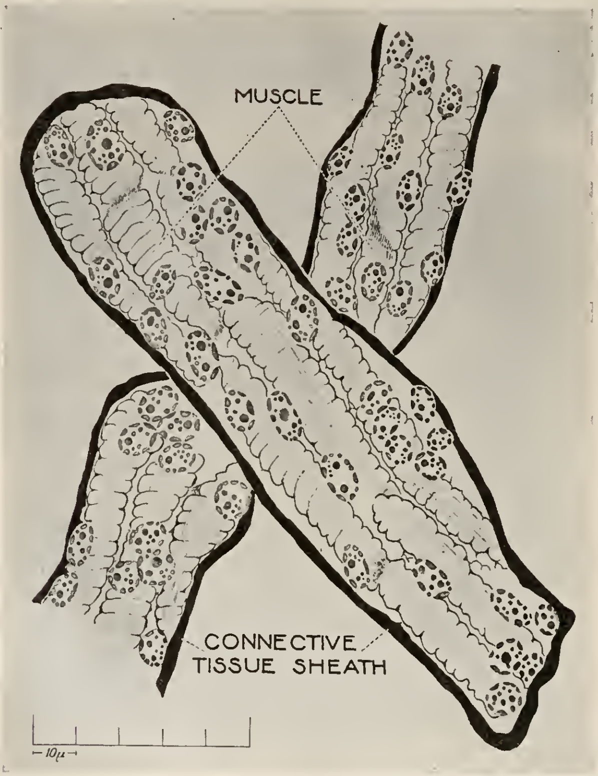

Figure 7. Longitudinal section (at point of crossing) of remnant of prothoracic glands of male adult *Leucophaea* one month after emergence. Note disappearance of glandular component, thickening of connective tissue sheath. Paraffin, 4 micra, Mallory azan.

Discussion

The description given in the preceding paragraphs requires a few words of discussion of the place the organ in question occupies with reference to corresponding (homologous and analogous) structures. There can be little doubt that the prothoracic glands of *Leucophaea* are homologous with those of the *Lepidoptera.* Both are located in the anterior thorax. As in *Leucophaea* the prothoracic glands of certain *Lepidoptera* are more or less branched, band-like structures without ducts (Toyama, 1902/03; Lee, 1948). In both, the paired glands are composed of epithelioid cell elements with densely packed nuclei and a small amount of cytoplasm.

The innervation of the prothoracic glands does not represent a pertinent criterion for their identification, since a considerable variation seems to exist even within the *Lepidoptera.* In this group of insects the prothoracic glands may receive fibers from the subesophageal ganglion, from the prothoracic ganglion, from the mesothoracic ganglion, and from the connectives between these ganglia (Lee, 1948). The glands of *Leucophaea* are innervated by fibers from the prothoracic ganglion, an observation which is not at variance with Lee's observations in *Lepidoptera*.

The prothoracic glands of *Lepidoptera* are morphologically well developed and are active as endocrine organs only in the immature insect; their presence has not been observed in the adult moth (Williams, 1948). In *Leucophaea* likewise the glandular component of the organ regresses after the emergence of the adult.

A feature characteristic of the prothoracic glands of *Leucophaea* is the presence of striated muscular tissue in the center of the organ. No such elements have been described in the corresponding organs of other insects with the possible exception of the "parenchymatous tracheal organs" of *Nepa* (p. 195). The physiological significance of this muscle is unknown. It is possible that it is instrumental in discharging the secretory product from the organ. This interpretation is suggested by the occurrence of muscular elements in exocrine glands, such as the Malpighian vessels of certain insects, likewise long, thin structures whose content is propelled towards the alimentary canal by a longitudinal muscle (Palm, 1946). Among vertebrates, contractile elements in the cytoplasm of the myoepithelial (basket) cells of certain glands (salivary, mammary, sweat, oral glands, etc.) also are thought to facilitate the expulsion of secretory products.

Another parallel exists in the occurrence of myoid cells (reticulum cells with striated fibrous elements) in the thymus of a number of vertebrates (see Bargmann, 1943). Their morphological similarity with the striated components of the prothoracic glands of *Leucophaea* is of interest in view of other features the prothoracic glands of insects and the vertebrate thymus appear to have in common (p. 195).

Whereas the homology between the prothoracic glands of *Leucophaea* and of *Lepidoptera* is reasonably well established, it is not always easy to recognize from the descriptions in the literature which of various organs in the head and thorax of different insects correspond to the prothoracic glands. Williams postulated that the "ventral glands" (Pflugfelder, 1938) of *Dixippus* (*Orthoptera*) are homologous with the prothoracic glands of *Lepidoptera*. In this connection it is highly interesting that Pflugfelder (1947) in a more recent publication extended his study of the ventral glands to include a number of insect orders, i.e., *Odonata, Ephemerida, Plecoptera, Saltatoria, Phasmida, Dermaptera, Blattaria, Mantodea,* and *Isoptera*. According to these data there exist in almost all lower *Pterygota* glandular organs of an endocrine character which, in their topography and histology, show many similarities. These phylogenetically ancient ventral glands are assumed to be derived from originally segmental organs whose function was excretory. In some cases the connection with the place of origin, i.e., the ventral epidermis, is still evident. More specifically, the ventral glands developed from the ectodermal canal of their precursor organs in the respective segment. During this transformation the lumen of the excretory duct gradually disappeared, a process which is indicated by the presence of a vestigial lumen in the ventral glands of *Plecoptera* and *Dermaptera*. The wall of the duct became the endocrine tissue.

It is of interest that a similar transformation from nephridial organs to glands of internal secretion occurred in certain crustaceans. The rudimentary antennal gland of the isopod *Asellus,* a serial homologue of the ventral glands of insects, is endocrine in nature (Pflugfelder, 1947).

Another recent publication must be considered in a discussion of the problem of homology of the prothoracic glands. Cazal (1947) described organs in *Aeschna* (*Odonata*) as "massifs ectodermiques intersegmentaires" which, at least with respect to their component situated in the posterior head region, seem to correspond to Pflugfelder's ventral glands. With the prothoracic glands of *Lepidoptera* and *Orthoptera* (*Leucophaea*), Cazal's organs have in common their occurrence in the thorax, their paired, elongated, irregularly lobated structure, and their histological appearance. Like the prothoracic glands of *Leucophaea,* the "intersegmental organs" of *Aeschna* consist of modified epithelial cells with scanty cytoplasm and closely packed nuclei and exhibit a typical cyclic behavior. A quiescent phase (after each molt) is followed by an active phase (preceding each molt), during which the nuclei show mitotic and pycnotic pictures. A "crisis" (crise cinétique) occurs in the adult which leads to the involution of the organ, comparable to that of the prothoracic glands of other insects, especially of *Leucophaea.*

An additional point of agreement exists between the observations in *Aeschna* and those reported in the present paper. The structure of the "intersegmental or-

TABLE I

	Antennal glands	1st maxillary glands	2nd maxillary glands	Thoracic nephridia		
				1	2	3
Crustaceans	Present, rudimentary, or absent (endocrine in Asellus)	Rudimentary, or absent	Present, rudimentary, or absent	Absent or present (Branchiura); without canal in Ostracoda	Absent or rudimentary (Ostracoda)	Absent or rudimentary (Ostracoda)
Onychophora	Rudimentary	Salivary glands	Present	Present	Present	Present
Diplopoda	Absent	Absent	Tubular glands	Absent	Absent	Absent
Chilopoda	Absent	Absent	Salivary glands?	Absent	Absent	Absent
Insects:						
Apterygota	Absent	Absent	Cephalic nephridia	Absent	Absent	Absent
"Lower" *Pterygota*	Absent	Corpora allata?	Ventral glands	Absent	Absent	Absent
"Higher" *Pterygota*	Absent	Corpora allata?	Salivary glands, spinning glands? prothoracic glands (Bombyx)	Prothoracic glands?	Absent	Absent

gans" according to Cazal is identical with that of the corpora allata of the same species. The striking similarity between these endocrine organs and the prothoracic glands in *Leucophaea* has been discussed (p. 189). Comparable observations in his material and a study of the literature led Pflugfelder (1947) to suggest that the corpora allata, like the ventral glands, may be derived from nephridial organs.

If we accept this derivation as correct, the ventral glands of the lower *Pterygota* (like the cephalic nephridia of the *Apterygota* and the second maxillary glands of crustaceans) can be considered as serial homologues of the corpora allata of pterygote insects, as well as of the antennal glands of crustaceans. No derivatives of thoracic nephridial organs are known to exist in the insect orders possessing ventral glands. In all holometabolous insects, in *Hemipteroidea*, in *Mantis*, and perhaps in *Blatta*, organs corresponding to the ventral glands of other insects studied by Pflugfelder are said to be absent. However, in some of these forms (*Lepidoptera, Hymenoptera, Blattaria*) organs situated in the thorax, the prothoracic glands ("hypostigmatic glands" of Toyama, "intersegmental organs" of Cazal) appear to take the place of the ventral glands. Furthermore, it is possible that a reinvestigation of the enigmatic "parenchymatous tracheal organs" in the thorax of the *Hemipteran Nepa* (Hamilton, 1931) may link these organs with the prothoracic glands of other insects.

The possible homologies of the prothoracic glands are summarized in Table I, which is based largely on Pflugfelder's data concerning the developmental history of nephridial derivatives in the *Articulata*.

With respect to *Bombyx*, Toyama (1902/03) showed that in the embryo the prothoracic glands develop from the second maxillary segment and, due to a shortening of this segment in the course of development, subsequently become located in the anterior thoracic region. If a corresponding derivation can be demonstrated in other forms possessing prothoracic glands these glands can be considered homologous with the ventral glands. However, it is quite possible that in certain insects the prothoracic glands may be shown to be derivatives of nephridia of the first thoracic segment, in which case they would be serial homologues of the ventral glands. The variability in the innervation of the prothoracic glands which may be supplied from ganglia of several segments (Lee, 1948; see also Pflugfelder, 1947) would thus be better understood.

The striking similarities in the development, structure, and life history of the ventral glands, the intersegmental organs, and the prothoracic glands suggest a correspondence in function (see Table II). It seems justified, therefore, to consider them as homologous organs whose endocrine function appears to be concerned with the control of developmental processes, both embryonic and postembryonic.

It has been mentioned before (p. 193) that the prothoracic glands of *Leucophaea* and their homologues also have some features in common with the thymus of the vertebrates; they are listed in Table III. The most interesting of these parallelisms are the involutionary process at the onset of sexual maturity, and the hastening of this regression by allatectomy [3] and hypophysectomy respectively.

The significance of such comparable traits is admittedly conjectural. However, it may be pointed out that there exist other structural and functional correlations between certain organs and organ systems of invertebrates and vertebrates, which

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[3] These observations will be reported in greater detail in a later publication.

TABLE II

	Occurrence	Location	Morphology	Histology	Life history	Histological cycles relative to molting	Function
Ventral glands	Lower *Pterygota* (Pflugfelder '38, '39, '40, '47)	Ventrocaudal head region	Paired, elongate, lobated, originally with duct	Epithelioid cells of ectodermal origin, occasional very large cells (Saltatoria); structure similar to that of corpora allata	Presence in immature stages (embryonic and larval); regression in adults: nuclear fragmentation and degeneration (pycnosis); no regression in termite workers and soldiers	Secretory granules in intercellular spaces; mitoses before (last) molt and nuclear fragmentation	Assumed endocrine (regulation of development); dependency on corpora allata
Intersegmental organs (massifs ectodermiques intersegmentaires)	*Odonata: Aeschna* (Cazal '47)	Posterior head, thorax	Paired, elongate, lobated; intersegmental invagination continuous with epidermis	Epithelioid cells, dense nuclei, little cytoplasm; structure identical with that of corpora allata	Presence in immature stages, regression in adults ("crise cinétique")	Mitoses and pycnoses preceding molts, quiescent period following molts	Assumed control of nucleoprotein metabolism, comparable to lymphoid elements (thymus)
Prothoracic glands (hypostigmatic glands, Toyama)	*Lepidoptera* (Toyama '02/03, Ke '30, Fukuda '40, '41, Williams '47, '48, Lee '48)	Prothorax, mesothorax	Paired, elongate, lobated or branched, without duct	Large epithelioid cells, little cytoplasm	Presence in embryos, larvae and pupae, absence in adults		Endocrine: source of growth and differentiation hormone(s) regulating molting, pupation, and adult differentiation
	Hymenoptera: Cimbex (Williams '48)	Prothorax			Presence in larvae		
	Orthoptera: Leucophaea	Prothorax	Paired, elongate, without duct; good nervous and tracheal supply; muscular component	Epithelioid cells, little cytoplasm, occasional large cells; structure like that of corpora allata	Presence in nymphs, regression in adults	Cyclic mitotic activity	Assumed endocrine; dependency on corpora allata
Parenchymatous tracheal organs (?)	*Hemiptera*· *Nepa* (Hamilton '31)	Thorax	Muscular nature, rich tracheal supply		Regression in adults		Unknown

TABLE III

Prothoracic glands (and equivalents)	Thymus
Location in posterior head and thorax	Location in neck and thorax
Derived from bilateral invaginations of ectoderm	Derived from bilateral epithelial ingrowths
Ducts originally present, later rudimentary or absent	Ducts reduced to rudiments during development
Segmental development	Segmental derivation (from several pharyngeal pouches)
Syncytial structure (*Platysamia*)	Syncytial reticulum
Lymphocyte-like cell components (*Bombyx*)	So-called thymocytes of lymphocyte type
Muscular components (*Leucophaea*)	Myoid cells
Maximum size before emergence of adult, involution in imago	Maximum relative size before puberty, involution after puberty (higher vertebrates)
Influenced by endocrine disturbances (involution hastened by allatectomy, *Leucophaea*)	Sensitive toward endocrine imbalance, for instance hypophysectomy
Endocrine function established in *Lepidoptera* (control of developmental processes)	Doubtful endocrine function related to growth and development

cannot be dismissed as superficial and accidental similarities, but which indicate similar principles of functional organization. Thus the intercerebralis-cardiacum-allatum system of insects is analogous to the hypothalamo-hypophyseal system of vertebrates (Hanström, 1941; Scharrer and Scharrer, 1944), the internephridial organs of the worm *Physcosoma* to the interrenal body (Harms, 1921), the x-organ of crustaceans to the thyroid, and the sinus gland of crustaceans to the paraphysis (Hanström, 1941). From these examples as from many others the concept evolves ever more clearly that the gap between invertebrates and vertebrates has in the past been magnified out of its true proportions.

SUMMARY

1. The prothoracic glands of *Leucophaea maderae* are paired band-like structures located in close proximity to and innervated by the prothoracic ganglion. The longitudinal axis contains striated musculature, a nerve and a trachea. The glands are well developed in nymphal stages, but regress in the imago.

2. In the nymph the glandular tissue consists of layers of dense nuclei surrounded by scanty cytoplasm. In their histological appearance the prothoracic glands strikingly resemble the corpora allata of the same species. The nymphal prothoracic glands exhibit a cyclic nuclear activity, characterized by a spurt of mitotic divisions during the intermolt period and by quiescent phases preceding and following each molt.

3. In freshly emerged male and female adults the prothoracic glands are still nymphal in appearance. Involution takes place during the first week of the adult stage. It manifests itself by a breakdown of nuclei and results in a reduction of the tissue bands to practically nothing except the muscular core.

4. The prothoracic glands of *Leucophaea* are considered to be homologous with the prothoracic glands of *Lepidoptera* and *Hymenoptera*, with the "ventral glands" of lower *Pterygota*, with the "intersegmental organs" of *Odonata*, and possibly with the "parenchymatous tracheal organs" of *Hemiptera*. They have certain features in common with the thymus of the vertebrates.

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OBSERVATIONS ON THE RESPIRATION OF AUSTRALORBIS GLABRATUS AND SOME OTHER AQUATIC SNAILS

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Trematode diseases are best eradicated, or at least reduced in their incidence, by interrupting the life cycle of the parasites in the intermediate hosts, that is, in the case of human infections, by instituting campaigns against the snails harboring the juvenile worms. This has been attempted to date by methods founded purely on empirical findings. It seems probable that a study of the physiology of the snails might yield important clues for the development of chemical means of control.

From a theoretical standpoint it appears likely that snails may be killed by the use of compounds interfering with the cellular respiratory mechanisms. As a preliminary to such work, a study was initiated of the respiration of some fresh water snails. We report in the present paper the results of our experiments on the normal aerobic respiratory physiology of *Australorbis glabratus,* the intermediate host of *Schistosoma mansoni* in the West Indies and South America. Included also are some data on the respiration of other snail species, most of them belonging to genera transmitting trematodes of man or lower animals.

MATERIAL AND METHODS

The following species of snails were employed:

1. Pulmonates

   Planorbidae: Australorbis glabratus, laboratory reared from Venezuelan specimens; *Helisoma duryi,* in part specimens freshly collected at Kenilworth Gardens, Md., in part laboratory reared specimens derived from this stock; *Tropicorbis obstructus* and *T. donbilli,* both laboratory reared from specimens collected in Texas.

   Lymnaeidae: Lymnaea stagnalis, collected from Mullet Lake, Michigan; *L. palustris* from Stemple Creek, Marin County, California; *L. obrussa* from a canal in the vicinity of Washington, D. C. These snails were used shortly after their arrival in Bethesda.

   Physidae: Physa gyrina, in part specimens freshly collected at Kenilworth Gardens, Md., in part laboratory reared specimens derived from this stock; *Physa* sp. specimens freshly collected in a creek near Bethesda, Md., and laboratory reared specimens derived therefrom.

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2. Operculates
   - *Amnicolidae: Amnicola limosa,* collected in a canal near Washington, D. C.; *Oncomelania quadrasi,* laboratory reared specimens derived from snails collected in the Philippines; *O. nosophora,* used some weeks after having been received from Japan.
   - *Viviparidae: Campeloma* sp., used shortly after having been shipped from Douglas Lake, Michigan.
   - *Melaniidae: Semisulcospira* sp., laboratory reared specimens, derived from snails collected in the Philippines.
   - *Pomatiopsidae: Pomatiopsis lapidaria,* freshly collected specimens from Fairfax County, Virginia.
   - *Pleuroceridae: Pachychilus* sp., specimens collected in Guatemala and kept prior to the determinations for months in an aquarium.
   - *Littorinidae: Littorina irrorata,* recently collected from Wicomico River, Maryland.

All species, with the exception of *Littorina,* are fresh water species; *Littorina* occurs in brackish water.

With the exception of the very few cases in which entirely freshly collected specimens were used, the truly aquatic snails were kept in balanced aquaria in a room with a minimum temperature of 21° C. During the summer months the temperature rose to nearly 30° C. The snails were abundantly fed with lettuce leaves and fish food. From time to time some powdered calcium carbonate was added to the water. Those species (*Oncomelania, Pomatiopsis*) that lead in nature a semi-aquatic life were kept in aquaria simulating as best as possible their normal habitat (see Ward, Travis, and Rue, 1947, for details); their chief food consisted of leaf mold.

Before the snails were used for the actual determinations, the water adhering to the specimens was removed with filter paper; they were then weighed on an analytical balance to the nearest mg. The standard temperature used in all experiments, with the exception of those in which the temperature influence was studied, was 30° C. This temperature was slightly higher than that reached by the aquarium water during the summer months and was chosen in order to allow an adequate control of the water bath. This temperature was well tolerated by all species employed.

The respiratory medium was, during the first months of the study, filtered river water. Later on tap water was employed; this was allowed to stand for a minimum of 24 hours in the laboratory to permit the chlorine to evaporate. No difference in respiratory rate was noticed in either type of water.

The respiratory exchange was studied by means of Warburg manometers. The number of snails used for a single determination, the amount of water introduced into the flasks, the size of flasks employed, and the interval between readings varied, depending upon the size of the snails. Up to 16 specimens of the small species, or the juveniles of larger species, were used for each flask. The total capacity of these flasks was about 17 cc., and 2 cc. of water were used as respiratory medium. Of the largest species, single specimens were studied in flasks having a total capacity of about 120 cc.; in these cases 6 cc. of water served as medium. In this latter case an equilibration period of one hour was necessary before the manometers could be closed while 20 minutes were sufficient when the smaller flasks were employed Depending upon the respiratory rate and the size of the flasks, readings were taken every 15 or 30 minutes for periods of two to four hours. The manometers were shaken with an amplitude of 4 cm. 100 times per minute. Most of the snails remained very quiet in the vessels; only the *Physa* species had a tendency to leave the water and to affix themselves to the sides of the flask.

In most cases the oxygen uptake alone was studied, the carbon dioxide being absorbed in the customary manner by means of 10 per cent KOH. In most series atmospheric air served as gas phase. In these series we were interested in the oxygen consumption of the snails in air saturated water, or, what amounts nearly to the same, that taken by pulmonates from the air itself. The question of the influence of varying oxygen tensions was studied only with *Australorbis glabratus.* In these experiments the gaseous atmosphere was changed by passing gases of known composition for a period of 20 minutes through the manometers. Details will be given in a later section.

The respiratory quotient was studied in some series of *Australorbis glabratus.* Since experience in other experiments had shown that the respiration of these snails stays fairly constant over long periods of time, a modification of Warburg's direct method was employed. The snails were first introduced into flasks containing no KOH and the change in manometer reading was followed for one hour or an hour and a half. The flasks were then removed from the manometers, KOH and filter paper were introduced into the center well and, after reequilibration to the temperature of the water bath, a second set of readings was taken for an equal period as above. The error due to possible changes in the respiratory rates is small since only such experiments were used in which the two sets of readings gave steady values. A few experiments in which either the first or the second set gave inconsistent readings were rejected. The snails used in these series were handled as little as possible; they were not dried or weighed in order to avoid injuries to the shell. This is a very important point since a pronounced carbon dioxide retention sets in very rapidly after the shell has been cracked. The readings taken in these series were calculated as mm^3. oxygen or carbon dioxide per one snail instead of being related to weight or surface area and the RQ was calculated from these values.

RESULTS

1. Relations between size and respiratory rate

The snails varied considerably in size due both to species differences and to differences in age of specimens of one species. This gave an opportunity to study the relation between size and rate of oxygen consumption insofar as both inter- and intraspecific comparisons are concerned. The experiments summarized in this section were carried out from May to August; there was no indication that during this time seasonal variations in oxygen consumption occurred. The determinations were in a few cases carried out with freshly collected specimens; in most cases well fed aquarium snails were employed. This point is important. It will be shown in a later section that the respiratory rate of snails declines very rapidly during starvation. It was even repeatedly noted that determinations carried out on Mondays yielded somewhat lower values than during the remaining days of the week, the

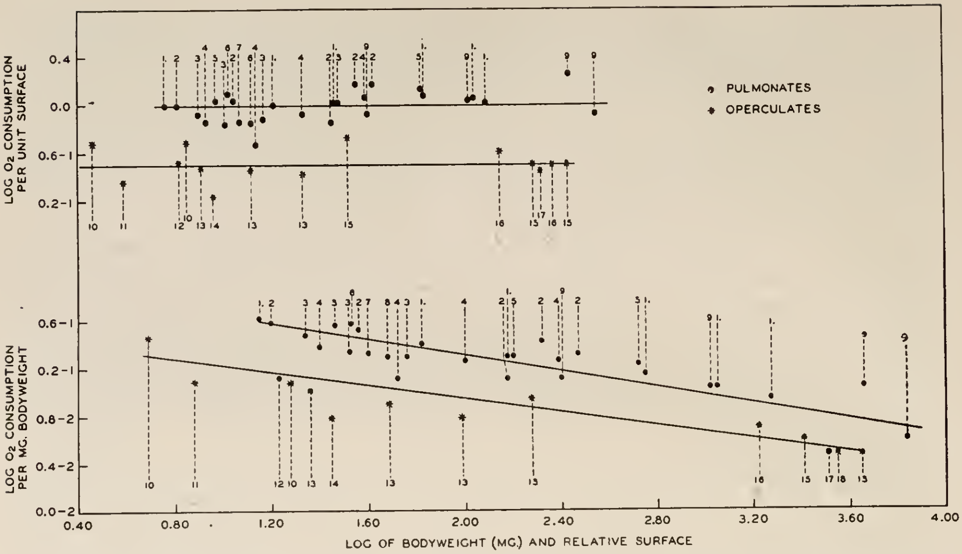

FIGURE 1. Relation between size and rate of oxygen consumption in various species of aquatic snails. Pulmonates: 1. *Australorbis glabratus,* 2. *Physa* sp., 3. *Physa gyrina,* 4. *Lymnaea palustris,* 5. *Helisoma duryi,* 6. *Lymnaea obrussa,* 7. *Tropicorbis donbilli,* 8. *Tropicorbis obstructus,* 9. *Lymnaea stagnalis.* Operculates: 10. *Amnicola limosa,* 11. *Oncomelania quadrasi,* 12. *Pomatiopsis lapidaria,* 13. *Semisulcospira* sp., 14. *Oncomelania nosophora,* 15. *Campeloma* sp., 16. *Pachychilus* sp., 17. *Littorina irrorata.*

TABLE 1

Respiration of Australorbis glabratus of various sizes at 30° C. Mean values and, in parentheses, extremes

Number of experiments	Number of snails	Weight of single snail mg.	Mm.^{3} oxygen consumed by 1 snail in 1 hour	Relative surface	Ratios of		
					Weight	Oxygen consumption	Surface
6	40	14 (12, 16)	5.9 (5.4, 6.2)	5.8	1	1	1
6	24	66 (55, 76)	16.8 (14.0, 20.2)	16.3	4.7	2.8	2.8
6	12	153 (122, 201)	30.3 (25.1, 38.4)	28.6	11	5.1	4.9
6	24	564 (525, 601)	81 (60, 95)	68.3	40	14	12
6	6	1137 (1000, 1288)	110 (84, 156)	109	81	19	17
6	6	1903 (1646, 2220)	164 (105, 284)	154	136	28	27

reason being that the snails had more or less exhausted their food supply over the week end.

The data summarized in Figure 1 are average figures from 4 to 12 determinations each, in most cases from 6 determinations. The sizes of the snails are also average values. The data for *Australorbis glabratus* (Table 1) may serve to illustrate these points and the variability introduced by them.

The data, as shown in Figure 1, prove three facts. First, the respiratory rate of pulmonates was without exception higher than that of operculates when snails of equal weight were compared. Second, the respiratory rate of both pulmonates and operculates was inversely correlated to the size of the specimens when calculated on the basis of weight. The slope of the straight lines around which the values of the two groups fluctuated was almost identical, indicating that about the same percentage decline in respiratory rate with increasing weight occurred in both groups. Third, it is quite apparent that straight horizontal lines resulted if the respiratory rates were calculated on the basis of relative surface ($\text{Weight}^{2/3}$) rather than weight.

Several reasons may be responsible for the deviations from the average straight lines which in some cases were rather marked. Although we tried to use for the experiments summarized in this section only well fed snails, it is entirely possible that not all species were equally near an optimum diet. Another point is that only the soft parts of the snails are actively metabolizing while our data are based on the complex soft parts plus shell. It was for various reasons not possible to determine in each experiment the shell weight and therefore no attempt was made to correlate the oxygen figures to the weight of the soft parts. Sixteen determinations of the shell weight were carried out at various times during the present experiments on various species; it was found that it varied between 11.2 and 25.4 per cent. It is, of course, also possible that the metabolic rate of various species is inherently somewhat different, even if nutrition and all other factors were exactly equal. Despite these deviations, the trend of the curves is convincing.

It should be mentioned as especially noteworthy that small and large specimens of a given species followed rather closely the surface law, as shown in Table 1 on the example of *Australorbis glabratus*. This is remarkable since in many other organisms juvenile specimens show a higher rate of metabolism than would be expected from this relationship. It is pertinent to mention that the australorbids used for these experiments were all taken from a single aquarium and that the experiments with them were all carried out within two weeks. We were therefore dealing with an exceptionally uniform material insofar as food and physical environmental factors were concerned.

2. *Influence of oxygen tension*

The experiments on the influence of oxygen tension on the oxygen consumption were carried out with *Australorbis glabratus* specimens of two sizes, small snails weighing 30 to 40 mg. each and medium sized snails weighing 300 to 400 mg. each. Fully grown specimens could not be used because the gaseous atmosphere could not be changed conveniently in the large flasks that alone would accommodate them.

The rate of oxygen consumption of the snails was first established at the oxygen tension of atmospheric air for a period of 1¾ to 2 hours with readings taken at 15 minute intervals. A gas mixture of known oxygen tension was then passed for 20 minutes through the flasks. After the manometers had been closed, the rate of oxygen consumption at the experimental tension was followed for two hours. The temperature was in all experiments 30° C. Twelve experiments were carried out for each of the two groups at each of the four experimental oxygen tensions, and each time new snails were employed.

The summary of all experiments (Table 2) indicates that the reactions of both size groups to changes in oxygen tension were very nearly identical. It is probably justifiable to assume that fully grown specimens would have reacted in an essentially identical manner. On the whole, there was little indication that the oxygen consumption was markedly influenced by changes in tension between 760 and 13 mm. Somewhere below this latter tension the consumption began to fall off rapidly; at a tension of 5 mm. only a small fraction of the normal amount was consumed. The data obtained prove conclusively that *Australorbis glabratus* belongs to the group of invertebrates capable of maintaining a more or less uniform rate of oxygen consumption over a wide range of tensions.

TABLE 2

Influence of oxygen tension on the oxygen consumption of Australorbis glabratus at 30° C. Mean values and, in parentheses, extremes

Series	O_2 consumption at 160 mm. Hg. tension mm.^{3}/1 gm./1 hr.	Experimental O_2 tension mm. Hg.	O_2 consumption at experimental tension	
			Mm.^{3}/1 gm./1 hr.	Per cent of 160 mm. value
A	296 (254, 318)	760	288 (250, 334)	98±8.0
B	140 (101, 177)	760	144 (97, 190)	104±3.1
A	255 (226, 270)	38	205 (186, 221)	81±2.0
B	161 (122, 187)	38	138 (99, 178)	86±2.3
A	311 (249, 416)	13	260 (234, 279)	85±3.2
B	163 (129, 183)	13	156 (110, 204)	95±4.1
A	246 (187, 310)	5	29.6 (8.2, 49.2)	12±0.4
B	177 (147, 262)	5	12.5 (3.8, 17.3)	7±0.7

Series A: Snails weighing 30 to 40 mg. each.
Series B: Snails weighing 300 to 400 mg. each.

3. *Influence of temperature*

The influence of temperature on the rate of oxygen consumption of *Australorbis glabratus* was investigated in the range of 0.3 to 41° C. The experiments were carried out with fairly small snails, the individual specimen weighing from 40 to 90 mg. Six snails were introduced into each flask and 12 experiments were conducted for each experimental temperature. New snails were used for each experiment. The respiratory rate of every lot was first established at our standard temperature of 30° C., four readings being taken at 15 minute intervals. The manometers were then transferred to a second water bath regulated to the intended experimental temperature. After equilibration the respiration was followed for a two hour period with readings taken at 15 or 30 minute intervals at the higher and lower temperatures respectively. The manometers were then transferred back to the original 30° C. water bath and a new set of readings was taken at 15 minute intervals in order to test whether during this recovery period the original level of oxygen consumption would again be reached.

The absolute values obtained in the various series during the initial 30° C. period varied somewhat, due probably to the differences in size between snails of the

TABLE 3

Influence of temperature on the oxygen consumption of Australorbis glabratus, averages and, in parentheses, extremes

Initial oxygen consumption at 30° C. mm.^{3} O_2/1 gm./1 hr.	Experimental temperature °C.	Oxygen consumption at experimental temperature		Oxygen consumption at 30° C. following experimental temperature			
				First hour		Second hour	
		Mm.^{3} O_2/1 gm./1 hr.	Per cent of initial value	Mm.^{3} O_2/1 gm./1 hr.	Per cent of initial value	Mm.^{3} O_2/1 gm./1 hr.	Per cent of initial value
151 (119, 186)	0.3	6.6 (3.7, 10.0)	4.3±0.25	58 (39, 74)	39±2.9	111 (73, 151)	75±6.9
222 (169, 260)	5.0	15.2 (11.8, 17.4)	6.9±0.34	131 (101, 162)	60±4.0	225 (185, 290)	102±4.7
208 (155, 256)	10.0	21.3 (16.7, 27.9)	10.2±0.16	204 (166, 253)	98±1.9		
184 (136, 224)	14.8	48.3 (40.0, 54.3)	27±1.3	178 (167, 196)	99±5.0	175 (150, 222)	97±4.9
179 (141, 234)	19.7	88 (76, 96)	50±2.2	185 (158, 214)	105±3.9		
175 (134, 200)	24.7	115 (94, 140)	67±3.5	168 (145, 188)	97±2.9		
193 (139, 228)	37.0	281 (226, 358)	148±7.0	193 (138, 260)	99±3.8		
225 (190, 282)	41.0	158 (111, 221)	71±3.7	152 (86, 248)	68±7.2		

various batches and to small differences in their nutritional state. These differences do not, however, interfere with an evaluation of the experiments since the figures found during exposure to the experimental temperatures and those obtained during the recovery period could be expressed in per cent of the initial value, thus eliminating any influence of these variations.

The oxygen consumption of the snails (Table 3) increased in the range 0.3° to 37° C. At 41° C., however, the animals were definitely damaged. Their respiratory rate decreased, and it did not come back to the original level during the recovery period. After the end of the recovery period, the snails were kept in beakers over night at room temperature and it was found that all were dead the following morning. The lowest temperature employed, 0.3° C., was also damaging. The respiratory rate increased only slowly after the snails had been transferred back to 30° C., and, after being kept over night at room temperature, about half the snails were dead. All other temperatures were well tolerated and the respiration returned during the recovery period to the pre-experimental value.

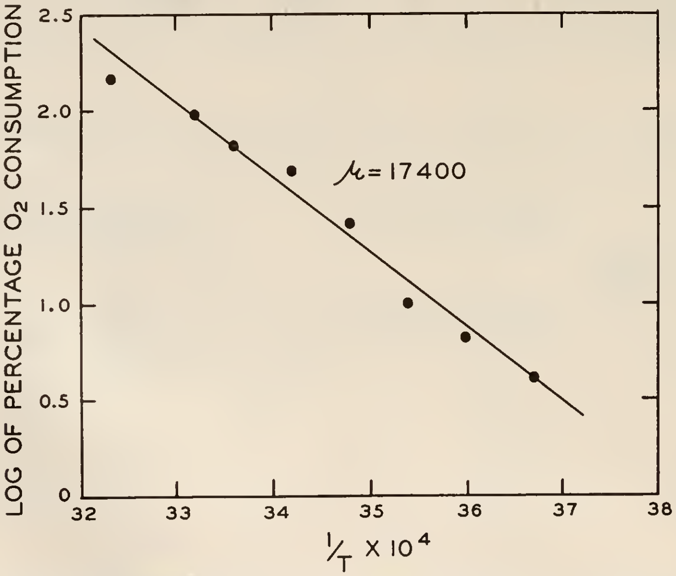

FIGURE 2. Temperature relationships of the oxygen consumption of *Australorbis glabratus* in the range 0.3° to 37° C. expressed according to Arrhenius' equation.

Using the percentage oxygen figures, the temperature relationship was then calculated according to Arrhenius' equation (Fig. 2) for the range 0.3° to 37° C. A single straight line was obtained and the μ value of 17,400 is entirely within the normal range.

Upon projection of the percentage figures on Krogh's (1914) normal curve a very satisfactory agreement to this curve was obtained (Fig. 3). Krogh's curve has been established only for the range 0° to 29° C. The points obtained in the present investigation beyond this range show an excellent fit to an extension of this curve. It would seem possible that this extension may have the same general applicability as the original curve.

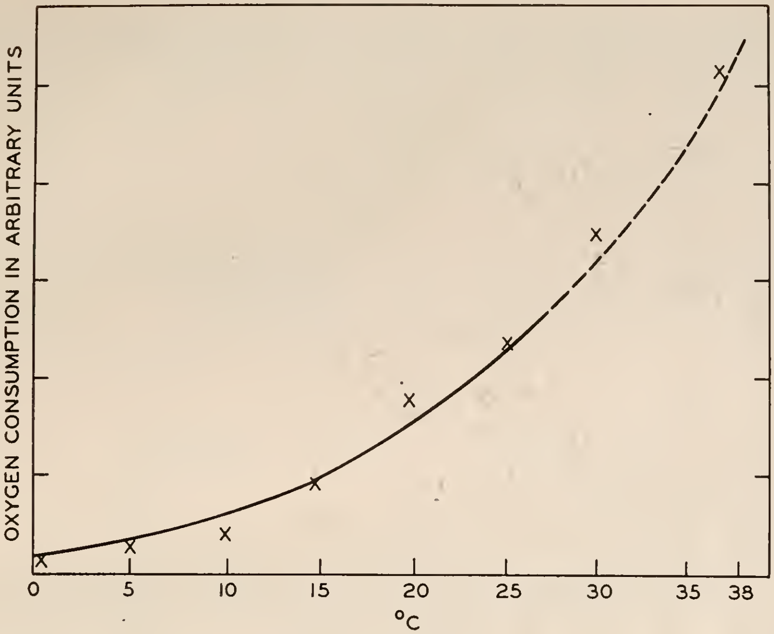

FIGURE 3. Projection of the percentage oxygen figures of *Australorbis glabratus,* taken from Table 3, on Krogh's normal curve and extension of this curve to 37° C.

4. *Influence of starvation*

The influence of starvation on the rate of oxygen consumption was studied with *Australorbis glabratus, Helisoma duryi, Physa* sp., and *Physa gyrina.* Of the three former species six groups of two snails each were employed while six groups of four snails each were used of *P. gyrina.* The snails of each species were selected as to uniformity in size. Each group was kept in a beaker with about 200 cc. water which was changed at frequent intervals and which was aerated by a slow stream of air bubbling through the water. The experiments were carried out during the summer months, the beakers being kept in a room without temperature control. The water temperature was, however, checked daily. It varied between 21.0° and 29.5° C.; the average temperature was about 27° C. The rate of oxygen consumption was determined for each group at the start of the starvation period, and after designated periods of starvation; the temperature during the actual determinations was 30° C.

The snails were weighed before each determination. The average loss in weight towards the end of the experiments was 18 per cent of the original weight in the case of *Physa* sp., 13 per cent both in *Australorbis glabratus* and *Physa gyrina,* and only 3 per cent in *Helisoma duryi.* Why this latter species lost relatively so little weight is not clear; it was the species that resisted starvation longest. Because this loss in weight would mask to a certain extent the decrease in metabolic level, if the oxygen values would have been calculated on the basis of the weight the organisms had on a specified day, the values are expressed in mm^3. oxygen/1 snail/1 hour.

Figure 4 shows that the various series ended after three to seven weeks starvation. When one snail out of a group died, the group was discarded and the whole series was completed when one of the snails of the last remaining group had died. Obviously then, our figures do not indicate the upper limit of starvation that the various species can endure at the temperature prevailing in our experiments. We are even hesitant to assume that all our snails died of actual starvation; the unavoidable repeated handling may well have hastened the end of one or the other specimen.

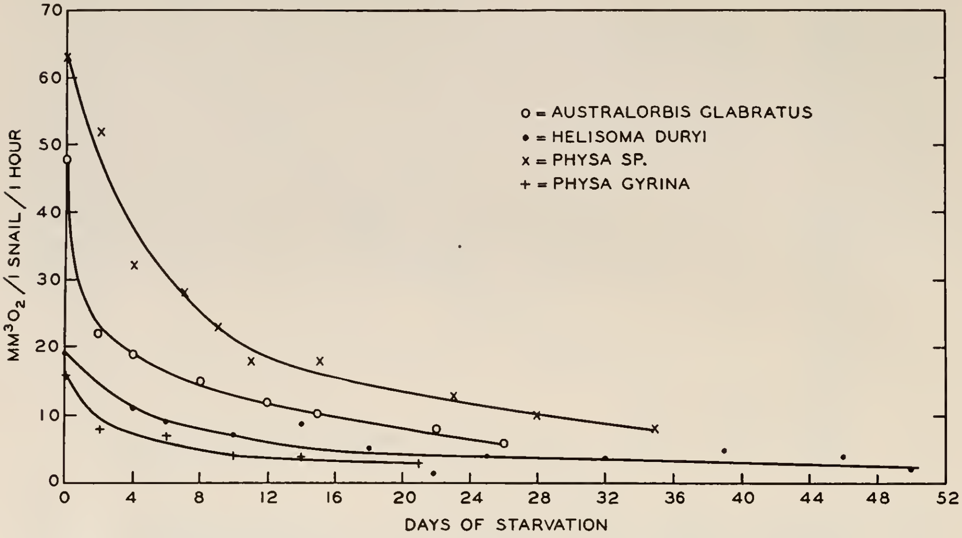

FIGURE 4. Influence of starvation on the rate of oxygen consumption of four species of pulmonate snails, absolute figures.

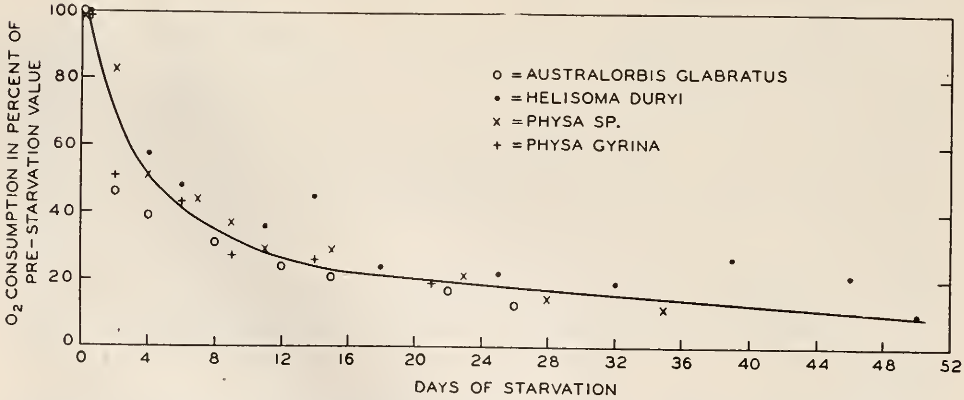

FIGURE 5. Influence of starvation on the rate of oxygen consumption of four species of pulmonate snails, percentage figures.

It is obvious (Fig. 4) that in all four species the rate of oxygen consumption declined sharply during the initial stages of starvation. Later the decline was much less marked, but no completely steady level was reached. In order to test whether the influence of starvation was noticeably different in the various species, the starvation values were then calculated in per cent of the initial values. This eliminated the differences in the absolute amounts due to the various sizes of the snails belonging to the different species. Figure 5 shows that the values thus obtained fit fairly well to a single curve; really large deviations occurred only during the first days of starvation. On the whole it is evident that the effect of progressive starvation was quite similar in the four species studied.

The respiratory quotient was studied only in the case of *Australorbis glabratus.* The snails were kept in these experiments in a room with an average temperature of approximately 25° C. but the actual RQ determinations were carried out at our standard temperature of 30° C. The points shown on Figure 6 are mean values of from five to 14 determinations each.

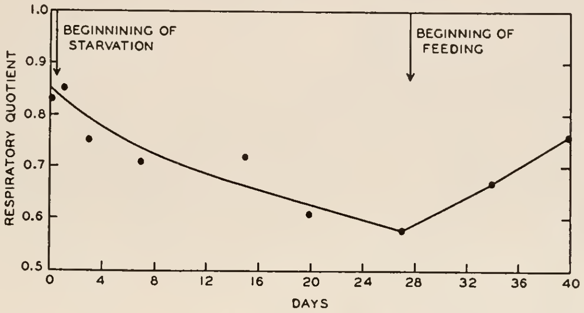

Figure 6. Respiratory quotient of *Australorbis glabratus* under the influence of starvation and subsequent feeding.

The respiratory quotient of well fed snails was around 0.85; the values found during the progressive stages of starvation fluctuated around the curve shown in Figure 6. A progressive lowering is evident and after three to four weeks starvation the surprisingly low value of 0.6 was reached. After 27 days inanition the snails were again fed and the respiratory quotient determined after one and two weeks feeding. It did rise during this period markedly but failed to reach the pre-starvation level.

Discussion

The present investigation has shown that aquatic pulmonate snails have consistently a higher rate of oxygen consumption than operculates of the same size. A definite reason for this difference could not be adduced; it is an illustration of the well known fact that animals with different organization frequently have different metabolic levels.

In both groups the respiratory rate was inversely correlated to the weight of the specimens, but remained more or less constant if referred to relative surface. This relationship held true both in intraspecific and interspecific comparisons and was especially close in the former case. The question of the relationships between body size and metabolic rate has recently been reviewed by von Buddenbrock (1939), Kleiber (1947), and Zeuthen (1947). While the surface law applies rather closely in the case of most vertebrates, the matter is more complex in invertebrates, or when the animal kingdom as a whole is considered. Large deviations are especially apparent in the largest and smallest organisms for which data are available. Insofar as molluscs specifically are concerned, Weinland (1918) found a positive correlation between surface and respiratory rate in *Anodonta,* while Liebsch (1928) denies such a relationship in terrestrial snails; he found a positive correlation between weight and respiratory rate. It should be pointed out, however, that the nutritional state of his snails was not uniform and that the size range of his specimens was appreciably smaller than that employed by us. An inspection of our data (Fig. 1) shows that within limited size ranges lines apparently showing a constancy of weight/O_2 relationship could be drawn but that the over all picture is distinctly against the validity of such a procedure. In view of the present results, it would be interesting to reinvestigate the terrestrial snails on a broader basis; it would be rather remarkable if they differed so fundamentally from the aquatic species.

It is true, however, that differences between terrestrial and aquatic snails have been reported also in other respects. It is thus a well established fact that the former show a pronounced dependency of their oxygen consumption on the oxygen tension (Thunberg, 1905; Dahr, 1927; Fischer, 1931; Harnisch, 1932) while many marine snails do not (Moore, Edie, Whitley, and Dakin, 1912; Raffy, 1933). *Australorbis glabratus* belongs, according to the present investigation, to this latter group. This, unquestionably, is due to the haemoglobin in its blood. Although the oxygen dissociation curve of the *Australorbis* haemoglobin has not yet been studied, it can be presumed to resemble that of the closely related *Planorbis.* The oxygen affinities of the latter's haemoglobin make it especially suited to procure oxygen at low tensions (Leitch, 1916; Borden, 1931).

The fact that *Australorbis* can hold its oxygen consumption at a normal level even at relatively low tensions may have a bearing on control measures. The application of some chemicals to snail infested waters results in the snails' burrowing into the mud and so escaping the direct action of the poison (W. H. Wright, personal communication). Although it is not known whether *Australorbis glabratus* specifically reacts in this way, it must be expected that snails with similar respiratory characteristics would not be easily killed by asphyxiation simply by being driven into oxygen-poor surroundings. Under very low oxygen tensions, it is true, the oxygen consumption is markedly lowered. It must be remembered, however, that snails in general are endowed with fairly well developed anaerobic functions (summary of the literature in von Brand, 1946).

The temperature relationships of *Australorbis glabratus* correspond to those commonly found in other invertebrates. The range of temperatures tolerated at least for the short periods employed in the experiments was rather wide. The variations in metabolic level encountered in this range may well have to be taken into consideration in control measures directed against this schistosomiasis-carrying species. Due to the lower metabolic level the snails will consume less food at lower temperatures than at higher ones. A poison, therefore, that acts via the alimentary canal may have to be kept longer at a given concentration in the cooler headwaters of a stream than in the warmer lower regions in order to insure that it reaches adequate concentrations within the tissues of the snail. It should be noted in this connection that according to Luttermoser (1947) in Venezuela at least "the only workable method for eradicating the snails was to eliminate them in the headwaters and to destroy them progressively downstream." Very likely, however, the temperature range in schistosomiasis-endangered river systems will not be quite so broad as the extremes employed in our laboratory experiments.

The influence of starvation on the oxygen consumption of *Australorbis glabratus, Helisoma duryi, Physa gyrina,* and *Physa* sp. was pronounced and quite similar in the four species. A progressive lowering in metabolic level occurred until the snails finally died. No steady rate of oxygen consumption was reached, the snails resembling in this respect starving warm-blooded animals (Krogh, 1916).

The respiratory quotient of *Australorbis glabratus* sank during starvation from an initial value of 0.85 to the surprisingly low level of around 0.6. Even lower values have been observed by Bellion (1909) in *Helix* and by Liebsch (1928) in several species of terrestrial pulmonates. *Helix* was studied towards the end of hibernation, that is, after having starved for a long time; Liebsch's specimens were probably at least semi-starving. The interpretation of the respiratory quotients of animals having calcareous shells is notoriously difficult; the following interpretation can therefore be only tentative. In view of the fact that the occurrence of glycogen has been demonstrated in *Australorbis glabratus* (von Brand and Files, 1947), it does appear probable that the relatively high RQ of well fed snails is due to the utilization of this polysaccharide. The glycogen reserve does not seem to last for a long time; soon values typical for fat and protein consumption are reached. The very low values found in the last stages may indicate either the new formation of carbohydrate from protein or, possibly, fat, or they may be due to carbon dioxide retention. The data at hand do not permit a decision between these possibilities.

The respiratory quotient of australorbids fed again after a starvation period of four weeks rose but failed to reach in two weeks the original level. Before starvation, the snails had been kept in a balanced aquarium; during and after the inanition period they were kept isolated in pairs in beakers. It is possible that they had in the aquarium some accessory food material at their disposal that was lacking in the beakers. But it is equally possible that during the recovery period a certain amount of carbon dioxide retention took place in connection with restitution processes on the shell. We gained at least in some cases the impression that the shells became brittle during protracted periods of starvation but we do not have quantitative data proving this point and emphasize that we do not consider it as more than a possibility. It may be mentioned that during hibernation, which of course corresponds to a starvation period, movements of inorganic substances between soft parts and shell likely occur in the case of *Helix* (von Brand, 1931).

Summary

1. A study of the rate of oxygen consumption of nine species of pulmonate snails and eight species of operculate snails showed that the pulmonates had consistently a higher metabolic level than the operculates if specimens of equal weight were compared.

2. In both groups, the intensity of oxygen consumption decreased with increasing size of the specimens if referred to unit weight, but remained about constant if referred to relative surface. The oxygen/surface relationship held true both in inter- and intra-specific comparisons and was especially close in the latter case.

3. *Australorbis glabratus* was able to maintain an approximately steady rate of oxygen consumption over a wide range of oxygen tensions.

4. The oxygen consumption of *Australorbis glabratus* increased with rising temperature in the range of 0.3 to 37° C., but 41° C. was lethal. The temperature relationship calculated according to Arrhenius' equation gave within the tolerated temperature range a straight line. A good fit to Krogh's normal curve was also obtained and an extension of this curve to a higher temperature range than used by Krogh is presented.

5. The intensity of the oxygen consumption of four species of pulmonate snails sank during protracted starvation first rapidly and later on slowly without reaching a steady level. The respiratory quotient of *Australorbis glabratus* sank during inanition to very low levels and rose only slowly after feeding was begun again.

6. The possible implications of some of the studied factors on snail control measures are briefly discussed.

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THE FUNGUS LAGENIDIUM CALLINECTES COUCH (1942) ON EGGS OF THE BLUE CRAB IN CHESAPEAKE BAY[1]

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In 1941 a parasitic fungus was first observed on the eggs of many blue crabs obtained from natural spawning areas in Chesapeake Bay (Sandoz, Rogers, and Newcombe, 1944). Dr. John N. Couch of the University of North Carolina kindly examined infected samples and recognized the parasite to be a new species, which he named *Lagenidium callinectes* Couch (1942).

Marked annual fluctuations in the commercial catches of the blue crab *Callinectes sapidus* Rathbun (1895) have taken place. The discovery of the egg parasite raised the questions of how it affects the development of the crab embryo, what percentage of the eggs of a crab may be infected, and how widely the parasite is distributed in Chesapeake Bay. Conceivably, such a parasite could constitute a serious biological factor limiting the production of crab larvae and causing, at least in part, yearly fluctuations in the commercial population.

Laboratory and field studies were carried out: (1) to ascertain the conditions of existence of the parasite in the individual crab eggs as well as on and in the egg mass (commonly called "sponge"); (2) to show how readily infection may be transmitted under certain conditions; (3) to indicate the effects of salinity and temperature on the survival and development of the fungus; and (4) to show the areas of Chesapeake Bay in which it occurs and the approximate degree of infection.

Acknowledgment is made to all persons who aided in this study. Special thanks are expressed to Professor John N. Couch of the University of North Carolina, who identified and described the fungus parasite; to Mr. John C. Pearson, Dr. Sewell H. Hopkins and Mr. R. Winston Menzel for certain crab collections; to Geo. W. Amory, Jr., W. J. Bradshaw, Jr., Chesapeake Crab Co., Costin Co. Inc., G. T. Elliott, Inc., V. S. Lankford, M. F. Quinn, and O. R. Mills for cooperation in making the collections possible; to Mrs. Ruth E. Allen for the use of illustrations; Mrs. Mildred D. Sandoz for helpful counsel; and especially to Dr. Sewell H. Hopkins for valuable criticism of the manuscript. This work was done under the direction of Dr. Curtis L. Newcombe, formerly director of the Virginia Fisheries Laboratory.

BIOLOGY OF THE BLUE CRAB

The blue crab, *Callinectes sapidus* Rathbun, occurs abundantly in Chesapeake Bay and provides the source for a major seafood industry in the Tidewater section of Maryland and Virginia. The annual production of the raw product of these crabs is valued at more than a million dollars.[3]

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[1] Joint contribution from the Virginia Fisheries Laboratory of the College of William and Mary and Commission of Fisheries of Virginia (Number 28), and from the Department of Biology of the College of William and Mary.

[2] Present address: Department of Zoology, University of Wisconsin, Madison, Wisconsin. This work was done in partial fulfillment of requirements for the degree of Master of Arts at the College of William and Mary.

Churchill (1919) made studies on the life history of this species in Chesapeake Bay. He found that the average blue crab lives two to three years during which time definite migrations take place throughout the bay. In the spawning season, which lasts from May to September of each year, large numbers of egg-bearing females are found in the waters at the mouth of the bay—in the vicinity of Cape Charles and in Hampton Roads (Fig. 1). The gravid female carries her eggs on four pairs of small abdominal appendages (pleopods). These appendages are provided with many hair-like filaments to which the eggs become attached by a glandular secretion when they are extruded from the oviduct (Fig. 2). Incubation is completed in about two weeks in Chesapeake Bay. An egg mass or sponge is estimated to contain about 2,000,000 eggs. The writer has observed that sponges vary a great deal in size, averaging 75 mm. wide, 50 mm. long, and 40 mm. deep. The bulk of so many eggs forces the folded abdomen (apron) away from the ventral side of the cephalothorax until it extends almost posteriorly. Observations have indicated that there is uniform development within a blue crab sponge (Lockhead and Newcombe, 1942), only 1 to 4 per cent of the eggs showing a retarded or undeveloped condition.

The approximate age of crab embryos can be determined by the color of the sponge. A new sponge is bright orange or yellow due to the large amount of yolk material in the egg. With development, the color of the sponge darkens to brown and finally black as the nutrient yolk is used up and pigment spots appear. Thus, age may be designated by three colors: Yellow, representing the first to the fifth day after eggs are deposited; brown, the sixth to the eleventh day; black, the twelfth to the fifteenth day.

Hatching releases zoeal larvae which are abundant in the plankton of the lower bay waters. After passing through at least five zoeal instars (Hopkins, 1944), a second larval stage, the megalops, is attained. There is a single megalops instar[4] which molts directly into the first crab stage. The young crabs begin to migrate up-bay or into the near-by rivers. Such crabs hibernate in these waters during the winter, then complete their development and mate the following summer. After mating, if not before, the females begin a migration to the natural spawning area in the vicinity of the Capes. Many arrive in the lower bay at the end of their second summer. They winter here and produce their eggs when conditions become favorable the following year. A large proportion of the females which mate late in their second summer may winter en route to the capes. Many of them produce their sponges the next summer before they reach the spawning grounds. This partially accounts for the large number of females with yellow sponges and the very few with dark sponges contained in commercial catches of the Egg Island-York Spit area (Fig. 1).

Adult male crabs do not make an extensive southward migration as do the

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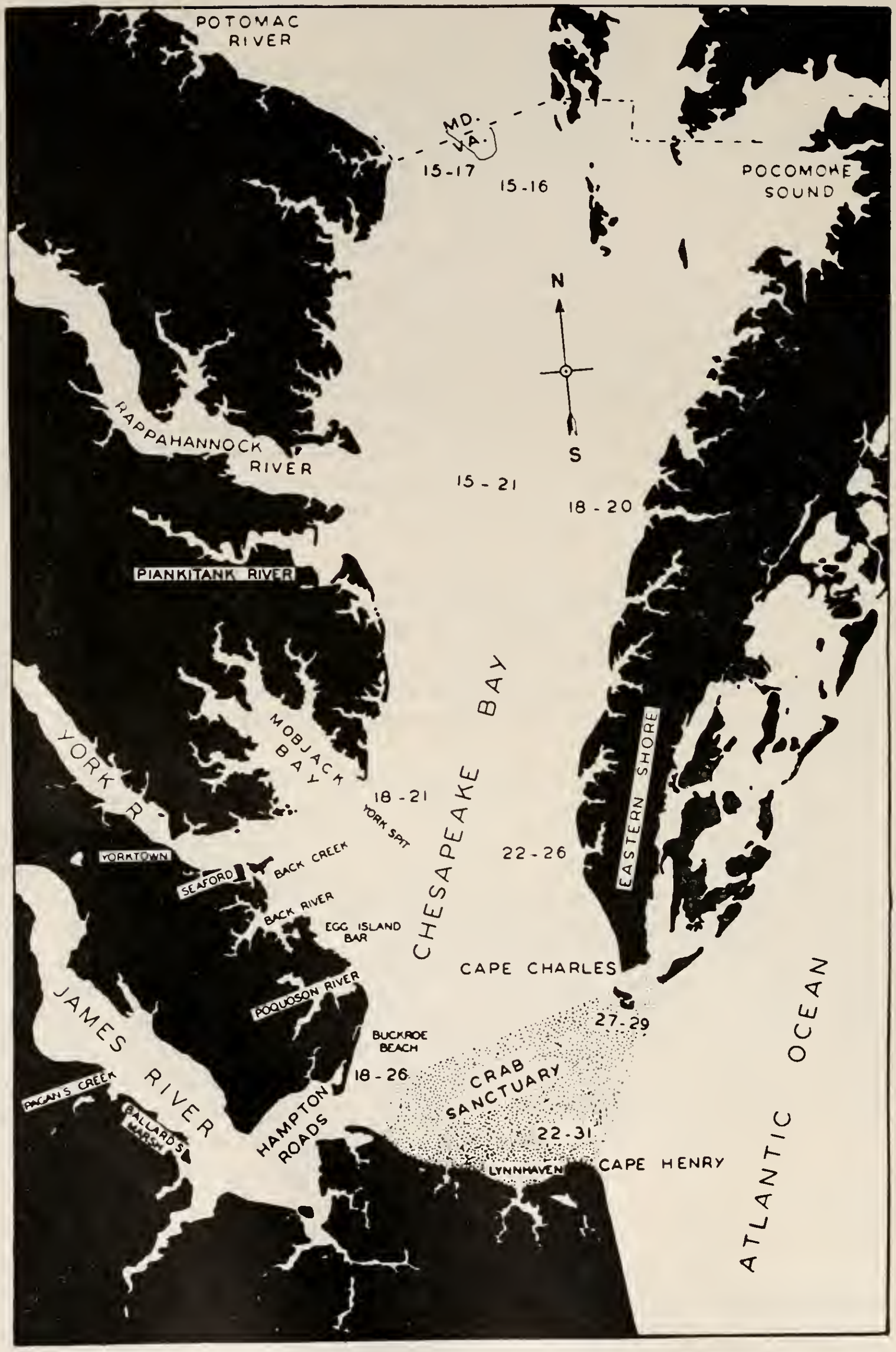
[3] For the period 1939–1943 the average annual production of raw product in Chesapeake Bay was 42,807,050 lbs., averaging an annual value of $1,327,882. *Fishery Statistics of the U. S.*, Fish and Wildlife Service, U. S. Dept. of Interior.

[4] M. D. Sandoz, in unpublished data on development of the blue crab, Virginia Fisheries Laboratory.


POTOMAC RIVER
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18 - 20
PIANKITANK RIVER
CHESAPEAKE BAY
MOBJACK BAY
YORK R.
18 - 21
YORK SPIT
EASTERN SHORE
22 - 26
YORKTOWN
SEAFORD
BACK CREEK
BACK RIVER
EGG ISLAND BAR
CAPE CHARLES
POQUOSON RIVER
27-29
BUCKROE BEACH
18 - 26
CRAB SANCTUARY
JAMES RIVER
PAGAN'S CREEK
BALLARD'S MARSH
HAMPTON ROADS
22-31
LYNNHAVEN
CAPE HENRY
ATLANTIC OCEAN

FIGURE 1. Lower Chesapeake Bay, showing the blue crab sanctuary and areas where blue crabs were collected. Average surface-bottom salinity records are given. (After Wells, Bailey, and Henderson, 1929.) Drawn by G. M. Moore.

females but remain in the rivers and bay waters along the entire length of the bay where they have matured.

The migratory habit of the blue crab endows Maryland seafood dealers with a greater proportion of the soft crab industry because the waters of Maryland and adjoining sections of Virginia are more heavily populated with immature crabs which undergo periodic moltings during their growth. The large population of mature hard crabs in the lower bay is responsible for the crab meat canning industry being located primarily at Hampton, Virginia.

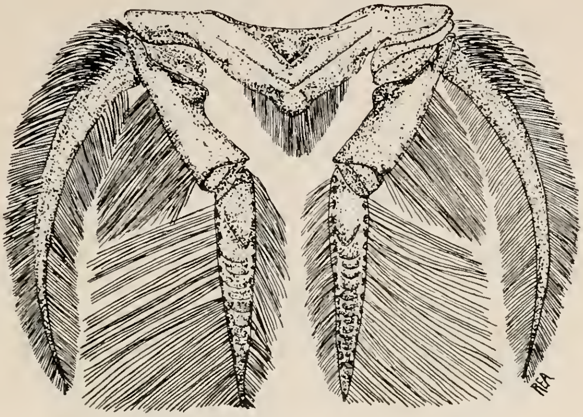

Figure 2. A segment from the abdomen of a female blue crab. Eggs are borne attached to the longer filaments of the endopodite. (Drawn by R. E. Allen.)

To protect the brood stock of blue crabs, the Commission of Fisheries of Virginia maintains a crab sanctuary (Fig. 1) at the mouth of Chesapeake Bay. Optimum conditions exist here for the development of blue crab eggs and crab fishing is prohibited in these waters during the spawning season. Examinations of egg-bearing crabs from the sanctuary in 1942 indicated that the fungus parasite, *Lagenidium callinectes* Couch, occurred there. This discovery aroused a wide interest among fishermen and conservationists and raised a question as to the value of protecting sponge crabs in the area. Furthermore, it pointed to a need for locating the waters where infection exists in order to determine whether the fungus is a general or localized parasite.

Characteristics of the Fungus

The description of the life history of *Lagenidium callinectes* Couch (1942) has been a valuable aid in this study. In his observations of the organism Couch found that when germination of the zoospore begins, a delicate germ tube is sent through the egg membranes. This tube grows rapidly into a network of branched mycelium that soon fills the entire egg (Fig. 3). From the mycelium, stumpy, thumb-like projections, or hyphae, pass through the egg membranes to the outside (Fig. 4). These hyphae quickly mature into sporangia which rupture and discharge new spores to continue the cycle of infection. When the nutrient material of the egg has been exhausted by the fungus, the mycelium appears to break up into heavy walled, resting cells that seem to be resistant to adverse conditions. However, neither germination of these cells nor a sexual phase of reproduction has yet been observed. Infected eggs soon give definite indication of being abnormal; they are opaque and dwarfed, the diameter becoming reduced from about 290 micra to approximately 231 micra (Fig. 4) (Couch, 1942).

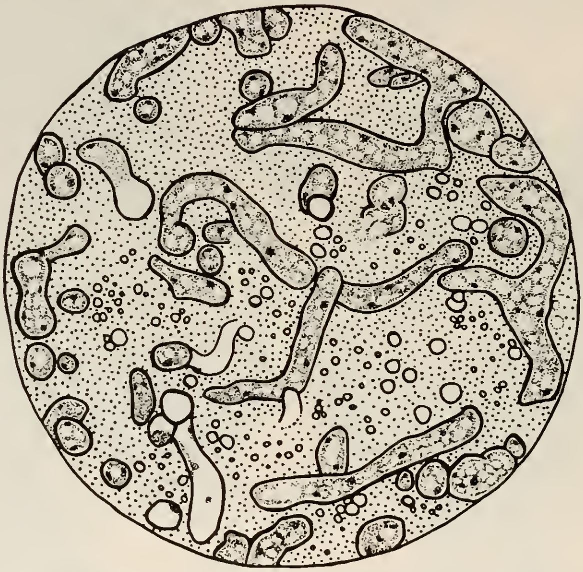

FIGURE 3. Cross section of a blue crab egg parasitized by *Lagenidium callinectes,* showing extensive internal mycelium (400 ×).

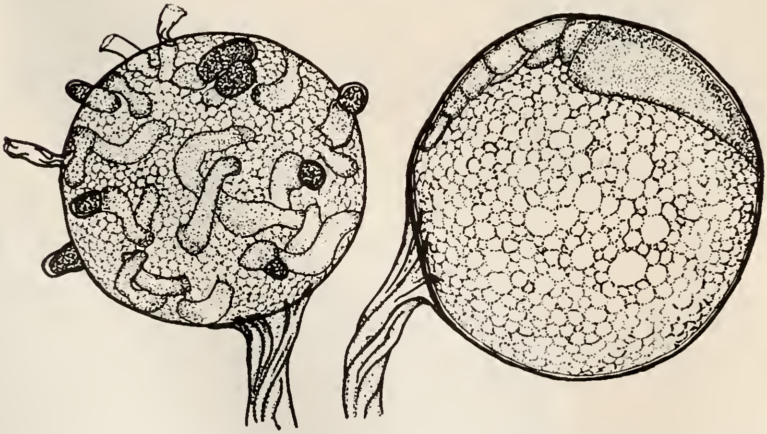

FIGURE 4. Two blue crab eggs from a single pleopod filament (200 ×). The parasitized egg (left) demonstrates 8 external hyphae and 3 empty exit tubes. Internal mycelium is seen through the transparent egg membranes. Parasitized egg shows reduction in size.

The several developmental stages as described by Couch were observed, and it has been possible to maintain the organism under laboratory conditions, thus providing a better understanding of how the parasite destroys the host egg. Among infected eggs collected from Chesapeake Bay and eggs infected under laboratory conditions, the number of external hyphae varies greatly; usually there are one or two on an egg, but frequently nine or more projections are observed at once.

METHODS

Studies of this parasitic fungus were carried out by random sampling of sponge crabs and by microscopic examination of the eggs. Lactophenol was used to clear the eggs and expose the mycelium. In this work the age of the sponge was designated by color: yellow, brown, or black.

Preliminary sampling up to and including 1943 indicated the waters of heaviest infection. Early in 1944 weekly sampling of various crabbing areas was begun. The samples, consisting of 20 to 25 sponges each, were preserved in 10 per cent formalin as soon as the commercial boats docked, which was only a few hours at most after the crabs were removed from the water. Collections were made in the Hampton Roads-Lynnhaven and Egg Island-York Spit areas. Relatively few sponge crabs are found north of York Spit.

To determine the extent of sponge infection, several methods were attempted before a satisfactory one was found. At first, eggs were taken at random from the outside of the mass and examined microscopically. A count totaling 500 eggs was made to estimate the percentage of exterior infection. Then about half the sponge was cut away and the procedure repeated, using eggs from the interior. It was found that infection did not penetrate to the interior, so observations were continued only on the peripheral portion of the sponge. Where infection was observed, several filaments were detached at the base and examined for the progression of fungus along the strand. These methods of computing degrees of infection involved a high probability of error in view of the enormous number of eggs per sponge. It was necessary, therefore, to abandon this plan of estimating the percentage of diseased eggs since it was impossible to count enough in every sponge to determine an accurate percentage.

Satisfactory results were obtained by setting up a standard based on visible areas of infected eggs. When the fungus has spread through many eggs in a given area of a sponge, the diseased portion in contrast to normal eggs assumes a brown color on yellow sponges and a grayish color on brown and black sponges. This is due to opacity of the eggs caused by the parasite. The following classification was adopted for differentiating the infected sponges in routine collections:

Slight—fungus present in microscopic examinations but no areas of infection visible to the naked eye.
Moderate—presence of visible areas of infection (which may be one or more) but less than half the sponge visibly infected.
Heavy—more than half of sponge periphery visibly infected, but with one or two small areas where infection has not become heavy enough to be seen.
Very heavy—a complete peripheral infection with no areas of healthy eggs visible.

From all the samples collected, four sponges were selected which demonstrated the different degrees of infection. From each pleopod of these sponges, 25 filaments were detached at the base and examined microscopically. Observations were made on the depth of fungus penetration, the general condition of interior eggs, and the posibilities of an appreciable hatch of larvae despite the exterior coat of infection.

Information on transmission of the fungus was obtained using the following procedures:

1. Several infected and uninfected egg-bearing crabs, selected from commercial catches at Seaford and Hampton, were placed together in aquaria.

2. Healthy and infected eggs from two different sponges were placed at opposite ends of porcelain pans and on opposite sides of large finger bowls. Running water from aquaria containing infected crabs was collected in pans into which normal eggs were then introduced. For controls, healthy eggs were placed in pans of water and females with sponges were placed in aquaria.

3. Infected and uninfected sponges in various stages of development were suspended in the York River near shore (Sandoz, Rogers, and Newcombe, 1944). A small cage (30 × 13 × 25 cm.) constructed of window screening on a wooden frame was used to protect the sponges and keep them afloat. Individual pleopods, detached from the sponge, were threaded with string near the base of the protopodite and fastened to hooks inside the cage.

To indicate how the salinity factor affects the fungus, a series of salinities ranging from pond water up to the approximate concentration of sea water was prepared, using pond water and salt extracted from York River water. In filaments selected for these salinity tests, the fungus had attacked all eggs within 2 or 3 mm. of the distal end; below this point eggs were developing normally. One filament was placed in each Petri plate of 50 cc. of water.

No other species of crabs has been observed with this infection, so studies were carried out to determine whether or not this parasite has a specific affinity for eggs of *Callinectes sapidus*. Strands of infected blue crab eggs were placed with the healthy eggs of several other species in Syracuse watch glasses containing York River water. The other forms of crabs included the oyster crab (*Pinnotheres ostreum* Say, 1817), the wood crab (*Sesarma cinereum* Bosc, 1801), the mud crab (*Neopanope texiana* Rathbun, 1900), and the spider crab (*Libinia emarginata* Leach, 1815), all of which are found in the area where infected blue crabs occur.

RESULTS AND DISCUSSION

Lagenidium callinectes Couch, a peripheral parasite. Microscopic examinations have shown that infection by *L. callinectes* is restricted principally to the periphery of a sponge (Fig. 5). In fungus infected crabs, all eggs from the distal end of the strand down about 3 mm. may become infected, but below this eggs are found to be normal.

Eggs lying in the interior of a sponge are packed closely together. The filaments, which are found only on the posterior side of the pleopods, vary in length from approximately 3 to 22 mm. (Fig. 2). The longer ones extend from the base of the pleopod while the short ones are at the tip. After eggs have been extruded, this variation permits none of the filaments to be buried within the mass. The volume of eggs is so great that the abdomen is pushed away from the cephalothorax and water flows freely around the outside of the sponge. The outer eggs of the mass serve as buffers for those on the interior, since commensals and parasites come in contact with these outer eggs first. Also, interior eggs lie closer together and do not seem to permit a rapid flow of water within the sponge, the interspaces only being large enough for water to seep slowly around the eggs. This movement of water is further aided by activities of the mother crab, such as vigorous jerking of her abdomen and frequent stirring of the eggs with her walking legs. *L. callinectes* gains a foothold rather quickly, but never seems able to penetrate to great depths within the mass. The female's habit of stirring the eggs with her walking legs may provide some opportunity for fungus spores to get into the interior, for in a few cases infection was found at a distance of 5 or 6 mm. down the filament. In only one sponge were infected eggs ever observed at the base of a filament and this filament measured but 13 mm. in length and was located at the outer end of the pleopod. Occurrence of infection inside the sponge, although uncommon, nevertheless provides positive evidence that conditions below the surface of a sponge are suitable for fungus growth. Hence, it seems that the outer eggs must act as a buffer providing a surface for attachment of organisms and a filter for the water that penetrates the sponge.

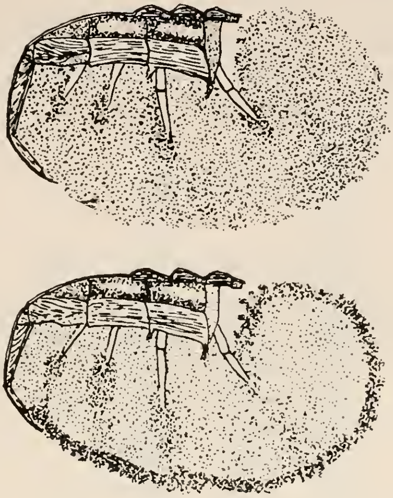

FIGURE 5. Egg masses of *Callinectes sapidus* Rathbun in longitudinal sections, showing a normal (above) and a diseased (below) condition with peripheral infection.

An infected area of a sponge increases rapidly in diameter while its penetration is much slower. A filament in such an area usually has all the eggs diseased for a length of from one to three millimeters at the distal end. Under the microscope the infection can be seen in various stages. In very heavy infections, the most distal eggs have had their nutrient material exhausted by the mycelium and resting cells have formed while the egg membranes may have started to disintegrate. Adjacent eggs to these have become very opaque and dwarfed and possess external hyphae and sporangia. The diseased eggs which are lowest on the strand are in the earlier stages of infection with only one or two empty spore cells on the outside; the internal mycelium is still developing, and very few or no external hyphae are visible.

In this study, examinations were made on disintegrating eggs at the distal ends of the filaments. If the disease destroys eggs rapidly it seems that many of the filaments would lose the cuticular covering which supports the eggs. However, no filaments were found where infection had progressed this far.

A small percentage of eggs destroyed by infection. In laboratory hatching experiments, uninfected egg-bearing filaments yield about a 90 per cent hatch (Sandoz and Rogers, 1944). Numerous empty egg cases observed on sponges removed from spawning grounds also indicate a high hatching percentage of uninfected egg masses in their natural environment. In the case of infected sponges, diseased eggs do not hatch but among the uninfected eggs on the same sponge it was found that the hatching percentage seems to remain high. Several sponges were examined which showed a very heavy peripheral infection beneath which the interior eggs were nearly all hatched out.

On an average-sized sponge of 2,000,000 eggs, about 10 eggs are distributed per millimeter of filament. The average length of all the filaments of a sponge is approximately 12 mm. In very heavy degrees of infection, if the distal 3 mm. of all the filaments were infected, there would be about a 25 per cent infection of the total number of eggs in the sponge. Of the total 2,000,000 eggs, 75 per cent or 1,500,000 eggs are in the interior and do not become infected but complete embryonic development and hatch normally. Moreover, a very heavy degree of infection occurs in slightly less than 25 per cent of the sponges; therefore it seems unlikely that *L. callinectes* can be regarded as a factor in the fluctuations of crab populations.

Older sponges more heavily infected. Samples of sponges in any age group show all degrees of infection; in view of which eggs must be susceptible to fungus spores throughout their developmental period. A large number of moderately infected egg masses with diseased patches on opposite sides, or on separate pleopods, indicate that a mass of eggs may be attacked by many spores simultaneously. In most of the sponges where slight infection was present, diseased eggs were found widely distributed over the periphery.

Figure 6 shows the incidence of infection by *L. callinectes* among sponges of different age groups throughout the summer of 1944. Less than 50 per cent of the yellow sponges showed infection while both brown and black sponges had a higher percentage of infection. This increase of infection in brown and black sponges is believed to be due to the eggs being older and, hence, exposed to infection for a longer time.

In examining the samples, consideration was given to the amount of infection present on each sponge. The various degrees of infection remained in almost equal proportions throughout the summer, thus indicating a fairly regular cycle for the parasite as regards the continuous infection of sponge crabs during the spawning season.

Zoeal larvae may become infected. In the laboratory, when infected eggs were present in the filaments, zoeae which had hatched normally often showed evidence of the fungus. It is believed that infection could not have occurred before hatching because the mycelium within 48 hours is able to fill an entire egg (Couch, 1942), and in all probability embryonic development would become disturbed within a few hours at most after penetration of the spore. The zoeae possess a very thin exoskeleton quite similar in appearance and thickness to the egg membranes which spores penetrate easily. It is more probable that zoeal infection occurs following a normal hatching of the egg. None of the larvae taken from plankton have ever been observed with fungus infection. Infected zoeae have been seen only in laboratory hatching pans, where the larvae must swim about in spore infested water. Under natural conditions larvae hatch from the sponge of the mother crab as she rests on the bottom in warm shallow water. They are positively phototropic and begin to move toward the surface. In this way the young swim away from the old sponge where infection may have contaminated the surrounding water with many motile spores. When the fungus invades the zoeae, the larvae soon weaken and become unable to swim. If such infection does occur in nature, this would explain the absence of diseased individuals from the plankton samples that we have studied.

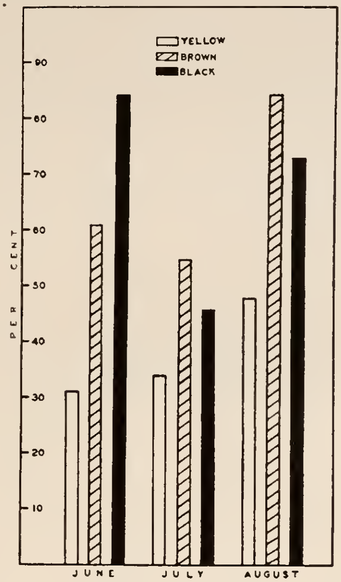

FIGURE 6. Percentage of yellow, brown, and black sponges infected by *Lagenidium callinectes*. Hampton Roads-Lynnhaven area. 1944.

Transmission of infection under experimental conditions. Laboratory cultures showed that transmission of infection from one egg to another is extremely rapid. Often an entire pan of eggs was destroyed by disease in three or four days, even when the first day showed very few infected eggs. In aquaria, healthy egg-bearing crabs quickly became infected when diseased crabs were introduced. In one case, water from an aquarium inhabited by a single infected female was used in a hatching pan which contained only normal eggs. Within two or three days *L. callinectes* was seen and a majority of the eggs soon became infected. In other experiments in which diseased and normal eggs were placed at opposite ends of a pan, the fungus was observed to infect the normal eggs after about two days.

Infected sponges which were suspended in the York River failed to hatch. During the experiment the number of infected eggs increased while the uninfected ones under the same conditions hatched normally, the zoeal larvae escaped and left behind their empty, transparent egg cases.

For experimental purposes normal eggs were usually selected from the Seaford catches where diseased crabs were seldom observed. There is no record of infection in the York River; consequently the chances of fungus having been introduced from the Seaford or York River waters are slight. Examination of controls never showed fungus growth.

Factors affecting the fungus. Laboratory experiments have demonstrated a wide salinity tolerance for this fungus. In all salinities, from 5 to 30 p.p.t., hyphal growth and spore formation proceeded rapidly. In fresh water during a two day period there was some development of external hyphae and a few small abnormal sporangia. During a two day period in salinities of approximately 15, 20, 25, and 30 p.p.t. there was such heavy growth that the eggs appeared to be enveloped in a fine white down. New eggs also became infected. In a salinity of 20 p.p.t. where the parasitic growth was extremely heavy, a typically infected crab egg was observed with seven sporangia, four exit tubes, and four hyphae, all visible from one side.

TABLE I

Percentage of sponges from Chesapeake Bay infected by Lagenidium callinectes Couch during the period 1942–1944

Location	Year	Number of sponges examined	Distribution of infection			Percentage of sponge infection
			Yellow	Brown	Black	
Lower Bay	1942	82	1	13	19	40
Lynnhaven Roads	1943	30	3	9	4	53
Lynnhaven Roads	1944	393	78	104	60	62
Lynnhaven River	1943	12	6	1	0	58
Lynnhaven River	1944	37	13	8	0	57
Hampton Roads	1943	15	1	8	4	87
Hampton Roads	1944	136	20	19	16	40
Ballards Marsh	1944	11	0	0	0	0
Seaford	1943	76	0	0	1	1
Seaford	1944	254	6	4	4	5.5
York River (at Yorktown)	1944	63	0	0	0	0
Rappahannock River	1943	6	0	0	0	0

L. callinectes can withstand sudden changes in salinity. The sponge crab used in this experiment was taken from Lynnhaven where the salinity is about 27 p.p.t. She was carried in a moist basket to the laboratory; eggs were cut from the sponge and placed in York River water (salinity 20 p.p.t.) for about an hour. When the salinity series was set up, the sponge filaments were transferred directly to pond water and salinities of 5, 10, 15, 20, 25, and 30 p.p.t. In no case except pond water was there apparent retardation in fungus growth. Development in salinity as low as 5 p.p.t. suggests that it may be possible for *L. callinectes* to become conditioned to very brackish water.

Low temperatures were observed to retard fungus development somewhat. This was first noticed in hatching experiments in 1942. When diseased eggs were placed in the refrigerator (15 to 16 degrees C.) fungus development and spore formation were delayed. This temperature, however, did not prevent sporulation and the spores continued to swim about, but their movement was sluggish.

Distribution of Lagenidium callinectes in Chesapeake Bay. Extensive samples of sponges collected during 1943–44 have indicated that *L. callinectes* is quite common in waters extending from Hampton Roads to Cape Henry (Fig. 1). However, the disease is not confined to these open areas. Samples from neighboring places also revealed the existence of fungus in inlets of the region. Samples from several miles up the Lynnhaven River showed a high percentage of fungus occurrence. In August, 1943, a sample from this river showed a 58 per cent infection; in July, 1944, another sample showed a 57 per cent infection (Table 1). In 1942, infected sponges were found in Pagan's Creek, a tributary of the James River. However, in August, 1944, a sample from Ballard's Marsh at the James River Bridge was not infected. In July, 1941, fungus was observed in a sample from Buckroe Beach, which represents the northerly limit of heavily infested waters.

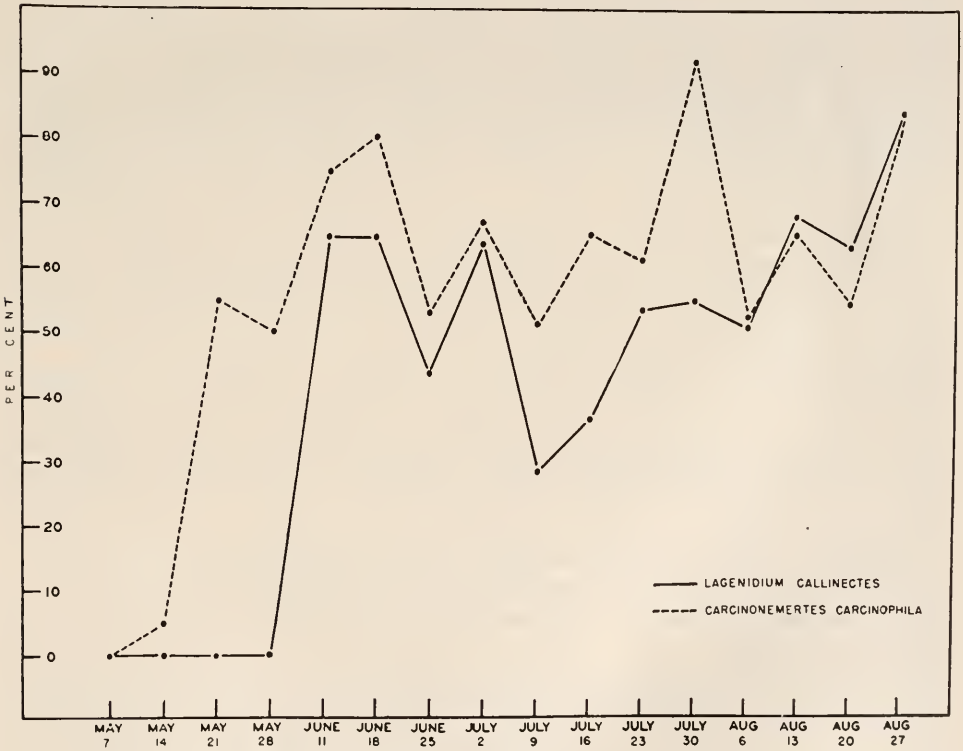

FIGURE 7. Percentage of blue crab sponges infected by the fungus *Lagenidium callinectes* Couch and by the nemertine *Carcinonemertes carcinophila* Kölliker during the summer 1944. Hampton Roads-Lynnhaven area. (Dates indicate first day of the week during which collections were made.)

In 1944, sponge crabs first appeared in commercial catches during the second week in May. The fungus was not present until a month later, the first record being taken from a sample collected on June 11 in which 13 out of 20 sponges were infected (Fig. 7). There seems to have been a simultaneous appearance of the fungus in both the Hampton Roads and Lynnhaven areas. This would indicate that the organism is well distributed throughout the region, spends a quiescent winter, and becomes active as soon as favorable conditions return. Egg-bearing crabs disappeared soon after August 31, 1944, until which time the fungus was present in more than 50 per cent of the specimens with a small increase during the last of August.

Samples from other regions of Chesapeake Bay have been examined. Throughout the Seaford area infection is uncommon. Several samples taken during June and August showed a 2 to 3 per cent infection. In one sample taken off Egg Island Bar at the mouth of Back River infection occurred in 45 per cent of the sponges. For this region the figure is high; however, Egg Island Bar is located in waters not far distant from Hampton Roads and the sanctuary where there is infection. In the York River at Yorktown, *L. callinectes* has not been found. One or two infected sponges have been taken from Mobjack Bay, Poquoson River, and the mouths of the York River and Back Creek. The degree of infection in most cases was slight. In these waters however, the majority of spawning crabs are yellow in color and are migrating toward the lower bay where hatching takes place. It is concluded that the general migration to the capes of spawning females is responsible for retaining the infection in this locality. When a female has completed spawning, the fungus probably ceases to live on that individual because hatching has depleted the food supply of the parasite. When the young crabs begin their northward migration, it is believed that the parasite remains behind since there is no evidence of an immature crab harboring the organism. The adult females probably die very soon after the completion of spawning so it is doubtful that spreading up the bay from the Lynnhaven area could occur by migration of infected females. Available information suggests that the fungus is localized in waters where female blue crabs hatch their eggs.

Occurrence of Lagenidium callinectes in other species. Laboratory experiments were carried out in an effort to infect eggs of *Pinnotheres ostreum, Neopanope texana, Libinia emarginata,* and *Sesarma cinereum.* The crabs used were all collected in the Seaford-York River region. Within two to five days, fungus was transmitted to eggs of the oyster crab (*Pinnotheres ostreum*) and the mud crab (*Neopanope texiana*).

Attempts to transmit fungus to eggs of *Libinia emarginata* and *Sesarmas cinereum* were unsuccessful, even though the latter remained alive for more than a week in the laboratory. However, previous hatching experiments with Libinia have never been successful.

Other organisms on the crab sponge. In addition to fungus, other organisms, either parasitic or commensal, are frequently found living on the sponge. These organisms, though quite common, seem to do very little damage to the eggs. Protozoan forms of *Carchesium* and *Ephelota* are often attached to the eggs in the peripheral portion of the sponge.

When fungus was first observed on the eggs of *Callinectes sapidus,* a hair-like growth longer than the diameter of a crab egg was noticed. Some eggs showed a profuse development of such filaments which at first were confused with the parasitic fungus. However, the filamentous growth later was recognized to be a *Chlamydobacterium* (sp.).

In 1944, while conducting crab studies at this laboratory, Dr. Sewell H. Hopkins found the parasitic nemertine *Carcinonemertes carcinophila* Kölliker (compare Humes, 1942) to be very abundant on the gills of the blue crab. This worm was likewise observed embedded in the sponge where it deposited its own eggs in a case entwined around the filaments. In these observations it was noticed that the nemertine and the fungus frequently occurred together (Fig. 7). The factors governing infection by *L. callinectes* and *C. carcinophila* appear to be quite similar, since the results show a corresponding periodic fluctuation of the two.

Significance of Lagenidium callinectes. From this discussion, *Lagenidium callinectes* has been found to be a peripheral parasite of the egg mass of the blue crab and the data obtained show that it is present in a large percentage of sponges (Table 1). When present, although it spreads rapidly among the peripheral eggs, penetration into the sponge is slow and rarely deep. Meanwhile, the healthy eggs of the interior, which in all cases represent at least three-fourths of the mass, continue their development and hatch normally.

This parasite, now evidently established within the spawning area, may possess the potential ability to destroy a great number of blue crab eggs. However, in the light of these observations, prevailing natural conditions seem to hold the fungus in check. It is known that the parasite has a fairly wide temperature and salinity tolerance, but the incubation period of the blue crab lasts only about two weeks which appears to be too brief a time for the fungus to work deeply into the center of an egg mass.

SUMMARY

1. The fungus parasite *Lagenidium callinectes* Couch has been observed to be a peripheral parasite of egg masses of the blue crabs of Chesapeake Bay.

2. Blue crab eggs are susceptible to infection in all stages of their development. Infected areas of a sponge are brown or gray in appearance, depending on the age of the eggs.

3. While the fungus spreads rapidly over the surface of the sponge, it penetrates the egg mass very slowly. Usually the depth of infection is not over 3 mm.

4. Infection is heavier in older sponges which are brown and black than in younger yellow ones, probably due to the longer exposure of older sponges.

5. Peripheral infection does not seem to retard the development of crab eggs in the interior of the sponge, which far outnumber the peripheral eggs. Not over 25 per cent of the eggs of a heavily diseased sponge are infected and only about 14 per cent of the crabs were found to be heavily infected. However, it was not unusual to find 80 or 90 per cent of the crabs in a sample to have some degree of infection.

6. Under laboratory conditions, transmission of infection from egg to egg of the same and different blue crabs is unexpectedly rapid.

7. Development of the fungus was observed to be abnormal in fresh pond water. In salinities from 5 to 30 p.p.t. development proceeded rapidly and indicated a strong tolerance of changes in salt concentration.

8. Frequently occurring on the peripheral eggs with *Lagenidium callinectes* are *Carchesium* sp., *Ephelota* sp., and *Chlamydobacterium* sp. *Carcinonemertes carcinophila* Kölliker is present and shows periodic fluctuations similar to the fungus.

9. Eggs of the oyster crab and the mud crab became infected with *L. callinectes* under laboratory conditions.

10. The Hampton Roads-Lynnhaven waters is the area in Chesapeake Bay where *L. callinectes* is most common. Only slight infection was observed north of Buckroe Beach.

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FACTORS INFLUENCING MOLTING AND THE SEXUAL CYCLES IN THE CRAYFISH [1]

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Introduction

There are two interesting phenomena associated with the periodic molting and the cycles of sexual functioning in crayfishes that have not been studied in detail. One of these interesting events is the delay in the spring molt of egg-carrying females until after the eggs hatch and the young crayfish leave the female; the other concerns the changes in the secondary sex characters in the male crayfish at the time of molt (Scudamore, 1942b). This investigation describes some of the factors influencing these two phenomena in the life cycle of the crayfish.

The delay in the spring molt of egg-carrying females has been observed by Van Deventer (1937), Tack (1941) and others. According to Tack (1941) the spring molt of males and non-reproducing females of the crayfish, *Cambarus immunis,* occurs about the middle of April in south central New York with most of the crayfish molting within the period of a few weeks. However, the females, which are carrying eggs at this time, do not molt until five or six weeks later. The reproducing females deposit their eggs in the fall shortly after mating and carry their eggs, attached to their abdominal pleopods, all winter. The eggs hatch about mid-May and the young remain dependent upon the female for a week or longer, while undergoing their first two molts. The egg-bearing female molts a few days after the young leave her pleopods.

The spring-molting period of males and non-reproducing females of the crayfish, *Cambarus propinquus,* also begins about the middle of April in central Illinois and lasts about three weeks (Van Deventer, 1937). However, the reproducing females do not deposit their eggs until early April. The eggs hatch about the middle of May and the young remain dependent for approximately another week. The egg-bearing females do not molt until late May or early June which is at least three weeks after the male spring-molting period.

This delay in molt of egg-bearing females is a protective adaptation, because molting earlier would result in the death of all the embryos. The mechanism producing this lag in the spring molt of ovigerous females has not been explained adequately. However, Hess (1941) reported a delay in molting of the seeded female shrimp, *Crangon armillatus,* as compared to non-seeded females. He also observed that removal of the embryos from seeded females shortened the period between molts and concluded that the factor, which inhibited molting, was apparently dependent upon attachment of the embryos to the female.

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[1] The author wishes to acknowledge the constructive criticisms and encouragement offered by Dr. Frank A. Brown, Jr., during the course of this investigation and to express his sincere appreciation to Dr. C. L. Turner for the use of unpublished field data.

The male secondary sex characters studied were the first pair of abdominal pleopods which are modified as gonopods for the transfer of spermatozoa to the annulus ventralis of the female during copulation. These gonopods have been described sufficiently by Turner (1926), Van Deventer (1937), Tack (1941) and others. Male crayfish are classified as Form I, II or "juvenile" on the basis of the morphology and function of the gonopods. Mature males with sexually-functioning gonopods are designated as Form I, and those with non-functioning gonopods, as Form II; immature males with non-functioning gonopods are classified as "juvenile." Most mature males change from Form I to Form II at the spring molt and revert to Form I during the summer molt in time to function during the fall mating season, remaining in Form I until the following spring. No satisfactory explanation of these changes in sexual form has been noted in the literature.

It is possible that sex hormones are involved in the delay of the spring molt of egg-carrying females and in the changes of sexual form of the male gonopods at the time of molting. However, there is no conclusive evidence for the presence of sex hormones in the crustaceans. As pointed out by Brown (1944) most of the proof for the presence of sex hormones is based on indirect results such as parasitic castration, radiation and regeneration experiments.

MATERIALS AND METHODS

Most of the laboratory experiments and some field observations were made on the crayfish, *Cambarus immunis* Hagen; but a few observations were made on *C. propinquus* Girard. The stock animals were kept in lead-lined tanks supplied with running tap water and were offered chopped earthworm or liver as food. The experimental crayfish were placed in individual finger bowls which were frequently refilled with fresh tap water and maintained at room temperature.

The eyestalks were removed by excising through the basal membrane with a sharp, pointed scalpel and coagulating the open wound with an electric cautery to control hemorrhage. Evidence of an approaching molt was obtained by sacrificing an animal and examining the anterior wall of the stomach for the presence of gastroliths. The pair of gastroliths were dried in an oven at 100° C. for 24 hours and weighed to determine gastrolith size. The carapace lengths were measured from the posterior margin of the cephalothorax to the tip of the rostrum.

The technique of inducing gastrolith formation and molting by removal of both eyestalks, developed by Brown and Cunningham (1939), Kyer (1942), Scudamore (1942a, 1947) and others, permitted (1) a study of secondary sex changes in male crayfish during winter molts induced by eyestalk removal as well as during normal spring and summer molts and (2) an investigation of the role of the eyestalks (sinus glands) upon molting in egg-carrying female crayfish.

FIELD OBSERVATIONS

Collections of *C. propinquus,*[2] made from a single locality during the spring and summer, illustrate the phenomena of the delay in the spring molt of egg-bearing females and the changes in sexual form of males (Table 1). On March 27th an ice jam had flooded a stream flat and, when the water receded, great numbers of crayfishes remained on the flat, either dead or in a dormant condition. All the males were Form I and had hard, calcareous exoskeletons. None of the females were bearing eggs. In this particular collection there were more females than males. According to Van Deventer (1937) males slightly exceed the females in number and, during the egg-bearing period, greatly out-number the females in the active population.

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[2] From the unpublished records of collections made by Dr. C. L. Turner in 1921 from Turtle Creek, Rock County, Wisconsin.

TABLE 1

Summary of molting and changes in the sexual cycles of the crayfish, C. propinquus, as observed in random field collections

Date	Total collected	Form I males		Form II males		Females		
		Number	Condition of exoskeleton	Number	Condition of exoskeleton	Number	Condition of exoskeleton	Number bearing eggs
March 27	270	109	Hard	0	—	161	Hard	0
April 15	76	68	Hard	0	—	8	Hard	7
April 24	156	124	Hard	0	—	32	Hard	26
May 1	48	46	Hard	0	—	2	Hard	2
May 13*	46	6	Hard	28	Soft	8	Hard	8
						4	Soft	0
June 3**	136	3	Hard	88	Soft Medium Hard	26	Hard	0
						19	Soft	0
July 6***	69	6	Soft	26	Hard	30	Soft	0
						34	Hard	0
Late July*** and August	Many	Many	Mostly hard	Very few	Hard	Many	Hard	None

* Spring molt of males and non-reproducing females.
** Spring molt of egg-bearing females.
*** Summer molt, both sexes.

On April 15th only eight females were secured in a collection of 76 crayfish which was made with a dip net without searching under stones. Seven of the females were bearing eggs upon their swimmerets. All the males were still Form I. Collections of April 24th and May 1st consisted chiefly of hard-shelled Form I males. The majority of the females were carrying eggs and were found hidden under stones. Apparently they had not moved from their hiding places or eaten for several days because an examination of their alimentary canals revealed no food. The few females not bearing eggs were moving around actively like the males.

By May 13th there were many cast-off exoskeletons lying in the margins of the shallow waters and practically all of these had come from Form I males but a few had come from non-reproducing females. Most of the males collected were soft-shelled and Form II, showing evidence of a recent molt. The four active females without eggs had molted recently. However, the eight females bearing eggs or young were concealed under rocks and had not molted.

Most of the males collected on June 3rd were Form II, indicating that they had completed the spring molt. The young crayfish, which up to this time had been clinging to the pleopods of the females, were in an advanced stage and many had left the females altogether. Many of the females were soft-shelled as a result of a recent molt following escape of their young, and nearly all of the exuvia lying in the shallow water were those of females that had carried eggs.

By July 6th most of the egg-bearing females had molted and some of the males had completed their second or summer molt. In late July and early August most of the males collected were Form I, indicating that the summer molt was completed. Some of the females apparently had undergone a second molt at this time and become sexually functional. In contrast to this observation, Van Deventer (1937) reported that adult females, which have borne eggs during the spring, undergo only a single molt.

Although it is difficult to delineate accurately the various events because of the length of time between collections, certain generalizations may be made regarding the life cycle of *C. propinquus* in southern Wisconsin. (1) Reproducing females deposit their eggs in early April and carry their eggs until the middle of May. The eggs hatch about mid-May and the young remain attached to the female for several days. (2) The spring-molting period of males and non-reproducing females begins after May 1st with many animals molting by May 13th and the remainder before June 3rd. (3) The spring molt of egg-carrying females, which is delayed until after the young have left the female, occurs during the month of June or about three weeks after the male spring molt. (4) The second or summer molt of most mature males and at least some females takes place during July and early August. (5) Most mature males change from Form I to Form II during the spring molt and from Form II to Form I during the summer molt.

Molting in Egg-Carrying Females

In order to determine the role of attachment of the eggs to the swimmerets in delaying molt, a number of egg-bearing female crayfish, *C. propinquus,* were placed in a large aquarium, closely simulating the natural environment, during the spring-molting period of males and non-reproducing females. The eggs were then removed from one group of females. Both those with eggs attached and those with eggs removed were given access to an abundant food supply. The egg-carrying females remained in their hiding places beneath stones in the aquarium, waving the mass of eggs attached to their swimmerets but not feeding. On the other hand, the crayfish, from which the eggs had been removed, soon began to move about freely, fed actively and molted within one or two weeks.

In experiments performed during the winter of 1941–42, twenty normal egg-carrying female crayfish, *C. immunis,* were placed in individual finger bowls and all of the eggs removed from the pleopods of ten of them; both eyestalks were extirpated from another group of twenty egg-bearing females, and the eggs removed from ten of these animals. The crayfish from each of the four groups were sacrificed (or died) and examined for the presence of gastroliths at various intervals of time. One eyestalkless crayfish in each group died of operative injury before sufficient time had elapsed for gastroliths to form (Scudamore, 1947) and so were not included in tabulating the results.

Only three of the normal egg-carrying crayfish were found to contain very small gastroliths and none molted (Table 2), even though these animals were observed for a period nearly three times longer than the eyestalkless crayfish (Table 3). The experimental period was long enough to permit gastrolith formation and molting when compared to normal pre-molt periods (Scudamore, 1947). Minute gastroliths have been observed in other normal crayfish during the winter, but their exact significance is not known. There was no essential difference in the results whether the eggs were removed or not. This experiment demonstrated that the mere removal of the eggs from the normal crayfish does not induce molting in a non-molting season.

Table 2

Influence of removing the eggs from the pleopods of normal egg-carrying female crayfish, C. immunis, upon gastrolith formation and molting from December to February

	Carrying eggs	Eggs removed
Number of animals	10	10
Average duration of experiment (days)	34.5	39.8
Number with gastroliths	2	1
Average weight of both gastroliths (mgm.)	0.25	0.06
Number molted	0	0
Average carapace length (mm.)	30.6	28.5

Table 3

Effect of bilateral eyestalk extirpation upon gastrolith formation and molting of egg-carrying female crayfish, C. immunis, from December to February

	Carrying eggs	Eggs removed
Number of animals	9	9
Average duration of experiment (days)	12.7	15.2
Number with gastroliths	9	9
Average weight of both gastroliths (mgm.)	44.80	54.19
Number molted	1	2
Average carapace length (mm.)	28.6	27.8

All of the eyestalkless crayfish had formed large gastroliths in 5–19 days after eyestalk removal and three of them had molted between 15 and 17 days after operation, even though winter is normally not a molting season (Table 3). The removal of the eyestalks resulted in gastrolith formation or molting whether the eggs were present or not. Although there was considerable individual variation, there was no significant difference in the rate of gastrolith formation in the two groups of eyestalkless crayfish (Table 4). The inhibition of molt seemed to be dependent upon both the presence of the eyestalks (sinus glands) and the attachment of the eggs to the pleopods of the female and not simply the attachment of the embryos to the female as concluded by Hess (1941).

Although the evidence suggests that the sinus gland molt-inhibiting hormone is responsible for the delay in the spring molt of egg-bearing female crayfish, there are a number of possible factors that may operate in maintaining the sinus gland activity until after the eggs hatch and the young leave the female, namely: hormonal, metabolic or nervous factors.

A female sex hormone elaborated in the ovaries or other tissues may cooperate with the sinus gland activity in the delay of molt. However, the lack of histological or experimental evidence for such glandular tissue (Brown, 1944) weakens this hypothesis. In this connection Turner (1935) reached the conclusion, on the basis of morphological studies, that the complete development of the annulus ventralis, a female secondary sex character, depends upon some ovarian tissue and the total absence of any testicular tissue. Yonge (1937) reported a cycle of histological changes in the oviducal epithelium and of secretion from the "cement" glands of the pleopods associated with egg-laying and attachment of the eggs to the pleopods of the lobster and suggested that, in the absence of nervous connections to the epithelium or the glands, the cycle of changes seemed to be controlled by hormones.

TABLE 4

Rate of gastrolith formation in eyestalkless egg-carrying female crayfish, C. immunis, during the winter

Day after eyestalk removal	Carrying eggs		Eggs removed	
	Length of carapace (mm.)	Weight of gastroliths (mgm.)	Length of carapace (mm.)	Weight of gastroliths (mgm.)
13	28.7	19.6	28.7	10.3
13	28.0	67.9	28.3	81.0
13	——	——	27.5	21.1
15	29.8	22.7	26.8	49.8
15	29.8	73.8	29.5	97.2*
16	23.2	40.2*	28.8	84.2
16	——	——	29.6	33.8
17	——	——	26.4	50.1*
19	31.5	69.7	25.0	60.2

* Indicates that animal molted.

The annual cycle of metabolic changes, which normally may initiate molting directly or through inhibition of the sinus glands, may be delayed in the egg-carrying females. The importance of metabolic factors is emphasized by the observation that egg-bearing female crayfish are largely inactive, hiding under stones and not feeding freely until after the young leave the pleopods. Furthermore, the egg-bearing females become active, feed and molt within a short period of time after the eggs are removed artificially during the male spring-molting period.

Finally, prolongation of the molt-inhibiting action of the sinus glands by impulses over nerve-reflex pathways produced by the presence of the eggs on the pleopods may explain the delay in molt. Welsh (1941) has demonstrated morphologically an innervation of the sinus glands of the crayfish from the "brain." Moreover, the tracts followed within the central nervous system of the crayfish by sensory impulses from stimulation of proprioceptors and sensory hairs of the abdominal pleopods were traced functionally by Prosser (1935), confirming the neurone paths first described histologically by Retzius (1890). These observations suggest the nervous pathways which may be involved in the reflex stimulation of the sinus glands. However, the fact that removal of only the eggs and not the eyestalks did not initiate gastrolith formation in the winter even in the warmth of the laboratory, suggests that some factor or factors other than possible pleopod-sinus-gland reflexes are involved.

Some combination of hormonal, metabolic and nervous factors seems like the most plausible explanation of this phenomenon. It is apparent that further experimentation is needed to establish the exact mechanism involved in the delay of the spring molt of egg-carrying females.

Molting and the Sexual Cycle of Males

The cycle of changes in sexual form of the first pair of abdominal appendages is illustrated by observations on molting in a single male crayfish, *C. immunis*, between April and September. This crayfish changed from Form I to Form II at the first or spring molt on May 15th and changed from Form II to Form I at the second or summer molt on July 15th.

In order to study the changes in sexual form of crayfish during the fall and winter, observations were made of changes in sexual form following molting of mature crayfish, *C. immunis*, induced by bilateral eyestalk extirpation and these changes were compared with those occurring at normal spring and summer molts. As shown in Table 5, all the crayfish became Form II after the artificially induced winter molts whether they were Form I or II before molt. The normal crayfishes changed from Form I to Form II at the spring molt and from Form II to Form I at the summer molt.. Enough Form II males for this winter experiment were obtained by selection from a large number of animals, because most of the males were Form I.

Table 5

Changes in sexual form of mature male crayfish, C. immunis, at the time of molt

Period	No. of animals	Original sexual form	Form after molt
Spring molt	12	I	II
Summer molt	12	II	I
Winter molt, induced by eyestalk removal	12	I	II
(November to March)	10	II	II

While studying spermatogenesis of the crayfish, Fasten (1914) found a seasonal variation in the size of the testes and in germ cell proliferation. The testes commenced active proliferation and increased in size in June, reached greatest activity and size in July, remained large in August with their tubules filled with spermatozoa, decreased in size in September, and remained small until the following summer. This seasonal cycle of changes in the testes of mature males is illustrated in Figure 1 together with the duration of the spring- and summer-molting periods, the seasons during which Form I and Form II mature male crayfish predominate, and the time at which copulation occurs.

The period of greatest testis activity (July–August) coincides exactly with the summer-molting period when males change from Form II to Form I. Copulation ensues a few weeks later at a time when the males have Form I gonopods and the testis tubules are filled with spermatozoa. During spring molts and during winter molts induced by eyestalk removal, when the testis size and spermatogenic activity are at a minimum, the male crayfish changes to Form II.

These observations offer circumstantial evidence in support of an hypothesis that variations in the amount of a male sex hormone, produced in the testes or other body tissues, are responsible for the changes in sexual form of the male gonopods at the time of molt. The cyclical release of such a hormone could be influenced by other internal or by environmental factors. The greatest weakness of this hypothesis is the lack of conclusive histological or experimental evidence for the presence of secretory cells within the testes. On the basis of morphological studies of the crayfish, Turner (1935) has considered that the development of aberrant secondary sex characters is largely dependent upon genetic rather than hormonal factors. However, the seasonal changes from Form I to Form II and the reverse obviously are not controlled genetically, since they occur in a single individual.

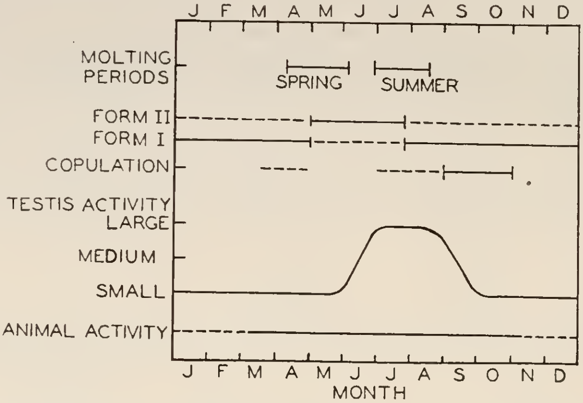

Figure 1. Diagram of certain phases in the life cycle of the mature male crayfish, *C. immunis*, demonstrating the relationship of molting periods, duration of and time of change to each male sexual form, period of copulation, periods of animal activity, and cycle of changes in testis size and spermatogenic activity. The curve of testis activity is based on the results of Fasten (1914). The solid lines (——) represent periods of predominant occurrence; broken lines (- - -), periods of occasional occurrence.

Proof for the existence of male, as well as of female, sex hormones must await histological evidence of secretory cells in the gonads or other tissues and the establishment of definite endocrine functions of these gland cells by surgical extirpation, implantation and injection of specific gland substances. However, observation and experimentation investigating the seasonal changes in the male gonopods represents a promising method for studying the problem of the existence of male hormones in crustaceans—a problem which is far from satisfactorily settled at this time.

SUMMARY

1. The phenomena of the delay in spring molt of egg-carrying females and of the changes in sexual form of males at the time of molt are described and illustrated by field observations on the crayfish, *C. propinquus*.

2. Removal of the eggs from the pleopods of egg-bearing females, *C. propinquus,* during the male spring-molting period results in an earlier onset of molting.

3. The delay in the spring molt of egg-carrying female crayfish, *C. immunis* and *C. propinquus,* is regulated by the action of the molt-inhibiting hormone of the sinus glands.

4. Various factors, that may operate to maintain the sinus gland activity until after the eggs hatch and the young leave the female, are discussed.

5. The changes in sexual form of male crayfish, *C. immunis,* at the time of spring and summer molts, and during winter molts induced by eyestalk extirpation are described.

6. Evidence is presented supporting an hypothesis that a male sex hormone, elaborated in the testes or other tissues, may regulate the cycle of changes in sexual form at the time of molt.

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PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1948

JULY 6

Action pattern of crystalline muscle phosphorylase.[1] SHLOMO HESTRIN.[2]

Degradation of glycogen, amylopectin, and amylose by repeatedly recrystallized muscle phosphorylase was studied. The reaction was found to be:

$$\text{polysaccharide} + \text{phosphate} \rightleftarrows \text{limit-dextrin} + \text{glucose-1-phosphate}$$

The limit-dextrin accounts for about 60 per cent of the weight of the parent polysaccharide in the case of glycogen and amylopectin, but must be a relatively minor reaction product in the case of amylose.

The phosphorylase limit-dextrin of glycogen was isolated and further characterized. The properties of this substance, in particular its ability to undergo a limited hydrolysis (24 per cent) by beta-amylase, are in accord with the view that it is derived from glycogen by a shortening of outer chains only, and that the final length of the shortened chains is probably three glucose units.

The phosphorylase limit-dextrin primes muscle phosphorylase and in suitable conditions can be shown to effect a shift in the equilibrium mediated by the enzyme. The findings thus further support Cori's theory that the primer is a stochiometric participant of the reaction.

Recrystallized muscle phosphorylase fails to degrade the beta amylase limit-dextrins of glycogen and amylopectin, and bacterial dextran. It is thus unable to cleave or by-pass an alpha 1–6 linkage and does not differ in this respect from potato phosphorylase as described by previous investigators. The enzyme may be regarded as a specific alpha 1–4 gluco-phosphorylase which acts on the 1–4 linkage only if the latter is terminal to a chain of sufficient length.

Crude muscle extract, in contrast to purified phosphorylase, converts glycogen and amylopectin almost quantitively to hexose phosphate. The factor in crude extract which mediates this effect is being studied further by Professor G. T. Cori and the author.

Vital Staining in ultraviolet and in white light combined. RUDOLPH KELLER.[3]

Living animals, such as salamander larvae, tadpoles, insect larvae, fish, daphnia, are first stained in the usual way by methylene blue, neutral red, alizarin, congo red or china blue and, afterwards, illuminated with luminescent dyes, such as primulin and aesculin, which make the daylight dyes visible in ultraviolet light.

With this method B. V. Pisha of this institution, using primulin, found that glands located in the distal end of the gut of daphnia showed a yellow greenish fluorescence. This, according to our former experiences, indicates the production of acid by the glands, which seems to neutralize the alkaline content of the gut.

In other experiments we stained with blue dyes such as aesculin (1 : 1000), positively charged in a biological medium, and administered after ten minutes, and a yellow dye uranin (1 : 10,000), also positively charged. We observed that the yellow dye rapidly displaced the blue one (which left the brood pouch of the daphnia at the neck) and the yellow uranin entered at the distal end of the pouch.

In further experiments we proceeded in the following way: To fish (Fundulus) in sea water in a vessel of 10 cc., three drops 1 per cent potassium ferrocyanide and two drops HCl 1/10 N were added. Later the animals were put into a solution of 1 : 800 thiazol yellow. The gills took up, first, in Prussian blue capillaries, only a little thiazol yellow. After killing the fish and adding new thiazol yellow, 1 : 800, to some gills on the slide, the space between some capillaries became strongly yellow greenish fluorescent, particularly in the neighborhood of injuries, while spaces between others appeared violet or scarcely stained.

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[1] This work was carried out in the Department of Biological Chemistry, Washington University School of Medicine, St. Louis, and was supported by a grant from the Corn Industries Research Foundation. The author is deeply indebted to Professor C. F. Cori for guidance in the conduct of the experiments.

[2] Hebrew University Travelling Fellow.

[3] Madison Foundation for Biochemical Research, New York.

The sulfhydryl metabolism of the beta cell and its relationship to the development of diabetes. ARNOLD LAZAROW.

Since glutathione protects rats from a diabetogenic dose of alloxan (Lazarow, *Proc. Soc. Exp. Biol. and Med.*, **61**: 441 (1946)), and since alloxan reacts with the sulfhydryl group of glutathione, and of protein, to give a new compound with an absorption spectra maximum at 305 mμ (Lazarow, Patterson, and Levey, *Science*, in press), it has been suggested that the glutathione, which is present in the pancreatic beta cell, normally serves to protect essential sulfhydryl enzymes from alloxan. It was further suggested that a low beta cell glutathione content could explain why alloxan is selective, but not specific, for beta cells.

It is estimated that the beta cells of man contain only 0.25 mgs. of glutathione [on the assumption that ½ per cent of the pancreatic weight (85 gms.) is islet tissue, and that the islet glutathione concentration is equal to that of the whole pancreas (60 mgs./100 gms.)]. Since insulin contains 12 per cent cystine, it is estimated that if all the cysteine contained in the glutathione of the beta cells of man could be incorporated into insulin, less than one milligram (19 units) of insulin would be formed. This is but a fraction of the daily insulin requirement of man.

Glutathione does not rapidly penetrate the cell membrane, for, on perfusion of liver, the glutathione is only slowly removed (Fabre and Simonnet, *C. R. de l'Acad. des Sci.*, **185**: 1628 (1927)). Following alloxan injection, the blood glutathione falls to near zero values, while the liver glutathione is only slightly affected. However, in spite of the large amount of glutathione in other tissues, the blood glutathione level has not returned to normal even after 6 hours (Leech and Bailey, *J. Biol. Chem.*, **157**: 525 (1945)). Thus cellular glutathione is not rapidly restored, and local depletions may take place. It is therefore postulated that the synthesis of insulin, in physiological amounts, may produce a local depletion in beta cell glutathione, and thereby render these cells more susceptible to alloxan or to other sulfhydryl inactivators, which may appear in the body.

Beta cell degeneration is also observed in (1) the pancreatic remnant following partial pancreatectomy, (2) following massive anterior pituitary hormone injections, and (3) after massive glucose injections. In all of these conditions, the beta cells are stimulated to an increased insulin production. It is further postulated that this increased insulin synthesis also sensitizes the beta cells to degeneration, because of a consequent local depletion in beta cell glutathione. If this theory of beta cell degeneration proves correct, then the glutathione metabolism of the beta cell will not only affect the etiology of alloxan, and other experimental diabetes, but it may also have an important bearing on the development of human diabetes.

JULY 13

A partial separation of the cytochromes of mammalian heart muscle. B. EICHEL, S. J. COOPERSTEIN AND W. W. WAINIO.

If successive amounts of sodium desoxycholate are added to an insoluble cytochrome complex preparation of mammalian heart muscle, the cytochromes can be partially separated. The desoxycholate is added at a concentration of 1 per cent and the undissolved residue in each instance is brought down by centrifugation at 20,000 × g for 1 hour. If 4 such successive fractions are prepared, the first fraction contains flavoprotein and cytochrome *c*, the second fraction contains cytochrome *c* and *b*, and the third and fourth fractions both contain cytochrome *b* and *oxidase*. However, fraction 3 has more *b* than *oxidase* and fraction 4 has more *oxidase* than *b*.

The absorption maxima in these preparations containing sodium desoxycholate were found at 416, 520, and 550 mμ for ferrocytochrome *c*, at 408 mμ for ferricytochrome *c*, at 429, 528 and 558 mμ for reduced cytochrome *b* and at 441 and 601 mμ for reduced cytochrome oxidase.

The identity of cytochrome *a* as a separate enzyme from cytochrome *oxidase* or a_3 (Keilin and Hartree terminology—*Proc. Roy. Soc. London,* **B127**: 167–91, 1939) is being investigated. The role of cytochrome *b* as a carrier for the dehydrogenases must be clarified with respect to its relation to the other cytochromes and to flavoprotein.

Alkaline phosphatase in demineralized mouse bones of different ages. ANITA ZORZOLI.

The general objective of this study was to determine the histochemical localization of the enzyme alkaline phosphatase in the tibia of normal mice during developmental life, during the period of growth and after growth of the bones had ceased. The bones were demineralized, without attendant enzyme inactivation, in a sodium acetate-acetic acid buffer at pH 4.55, sectioned and incubated with sodium glycerophosphate according to the method of Gomori.

Prior to the 15th day of gestation, the tibia was entirely cartilaginous and was completely devoid of enzyme. Phosphatase first appeared in the connective tissue surrounding a localized region of cartilage which was destined to become a center of ossification. Once the typical histological changes had begun, the enzyme appeared in the cartilage and was located in the nuclei, cytoplasm and to a slight extent in the matrix. Shortly later the bone salts became evident. It was interesting that their appearance was always preceded by that of the enzyme. With the spread of the processes of ossification the enzyme increased in amount and distribution.

In the early post-natal bones where rapid growth occurred, enzyme was prominent in the epiphyseal growth zone. The outermost cartilage cells of this region were small in size and were always phosphatase free. The adjoining cells, arranged in the form of columns parallel to the long axis of the bone, contained enzyme which increased in concentration with proximity to the hypertrophic zone. In the hypertrophic zone which was one of great activity, phosphatase occurred in both cells and cartilage matrix. It also appeared faintly in the calcified spicules and the newly formed bone. The osteoblasts were strongly phosphatase positive while osteoclasts were never observed to contain enzyme.

With increasing age the growth processes declined and the number of enzyme-containing cells of the hypertrophic zone decreased while phosphatase-free matrix increased. This change was already evident at 5½ months of age and by 13 months only a few scattered cells remained.

The order of amino acids in silk: an application of isotopic derivative technic. MILTON LEVY AND EVELYN SLOBODIANSKY.[1]

The principles described by Keston, Udenfriend and Cannan (*J. Am. Chem. Soc.* **68**: 390, 1946) are applicable to complex mixtures of amino acids, dipeptides and higher peptides as present in partial hydrolysates of silk. We have estimated the peptides of alanine (A) and glycine (G) in hydrolysates produced by the action of concentrated HCl at 39°. Thus, in a 48 hour hydrolysate the per cent of the total nitrogen in each form was: G, 12.9 per cent; A, 10.5 per cent; AG, 27 per cent; GA, 8.3 per cent; and GG, 1.8 per cent. Random arrangement of the 42.3 per cent G and 28.2 per cent A in our sample would have led to a maximum of 12.3 per cent AG. The arrangement cannot therefore be random. It is suggested that a unit of silk structure may be –G–X–A–G–A–G–X–. In this structure two AG's are possible for each GA. No GG is possible. X stands for any other amino acid. Further analysis of complete hydrolysates indicates in per cent of the total nitrogen the following amino acids: Serine, 9.24 per cent; glutamic acid, 1.07 per cent; aspartic acid, 1.54 per cent; hydroxyproline, 0.05 per cent. These analyses were done using the isotope derivative technic with separation by paper chromatography (Keston, Udenfriend and Levy, *J. Am. Chem. Soc.,* **69**: 315, 1947). It is noted that G, A and Serine are in the ratio of 9,6,2 and that glutamic and aspartic are in the ratio of 2:3.

The radioactive isotopes used in this work were supplied by the Clinton Laboratories on Allocation from the U.S.A.E.C. The work was supported by the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.

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[1] Stanley Tausend Foundation Fellow.

JULY 20

On the specificity of cholinesterase. KLAS-BERTIL AUGUSTINSSON.[1]

It has been demonstrated by different groups of investigators that the cholinesterase activities of various sources are not identical. A hypothesis has been proposed that two types of acetylcholine splitting enzymes exist.

There is no doubt, however, that many of the differences among the cholinesterases of various tissues cannot be accounted for by the fact that two types exist. This has been demonstrated in experiments with enzyme preparations from tissues and body fluids of various animals, vertebrates as well as invertebrates. A full account of these experiments has recently been published (Augustinsson, *Acta physiol. Scand.,* **15**: Suppl. 52, 1948).

Cholinesterases are defined as esterases which split choline esters at a higher rate than other esters; the specificity is not an absolute one. These esterases are regarded as a family of related enzymes with widely divergent properties. The following classification is based on the activity-substrate concentration relationships for the enzymic hydrolysis of acetylcholine.

Group I is characterized by the inhibition of cholinesterase activity at high acetylcholine concentrations; optimum activity at about 3×10^{-3} M acetylcholine. This group includes the cholinesterases of the nervous system, muscles, electric organs (Nachmansohn), erythrocytes, *Helix* blood, snake venom (Zeller). The enzymes of Group II follow the Michaelis-Menten formulation, which means that their activities are maximal only at infinite substrate concentration. This group includes the choline-ester splitting esterases of certain sera (e.g., man, horse), dart sac (*Helix pomatia*), and pancreas (Mendel). The properties of the members within each group may then differ in certain·other respects.

The optimum conditions in the hydrolysis of acetylcholine are *a priori* not identical with those prevailing in the hydrolysis of other esters. When, for instance, the substrate concentration is arbitrarily chosen in the enzymic hydrolysis of a choline ester or a non-choline ester, the optimum conditions are not the same as those of the hydrolysis of acetylcholine, the rate of reaction may be lower, the same, or even higher than that of the acetylcholine hydrolysis.

Effect of anticholinesterases on conduction. DAVID NACHMANSOHN.

In 1942 the theory was proposed that the release and removal of acetylcholine are intracellular processes necessary for the conduction of the nerve impulse. This idea was based on a great variety of facts obtained by the study of the enzymes connected with acetylcholine metabolism, and their correlation with the electrical manifestations in conduction.

If the rapid removal of acetylcholine is necessary for the propagation of the impulse, anticholinesterases should block conduction. This could be shown with eserine and other inhibitors of cholinesterase (*J. Neurophysiol.,* **9**: 9, 1946). In this case, inhibition of the enzyme and block of conduction are reversible.

Two years ago, this theory was assailed by several investigators based on observations with a new anticholinesterase, diisopropylfluorophosphate (DFP). This compound inhibits cholinesterase irreversibly. All the objections raised, however, and the apparent difficulties have been overcome, and the necessity of cholinesterase for conduction has been demonstrated conclusively.

Contrary to the original assumption, the irreversible inactivation of cholinesterase by DFP is not an immediate process but depends on a number of controllable factors. A striking parallelism has been established between the rate of irreversible abolition of conduction and that of irreversible inactivation of cholinesterase. This has been shown as a function of time as well as of temperature. The necessity of the enzyme for conduction has been established on a great variety of different types of nerves, and on striated muscle suggesting the same role of acetylcholine in all conductive mechanisms throughout the animal kingdom (*J. Neurophysiol.,* **10**: 11, 1947). In no way is it possible to dissociate cholinesterase activity and conduction. Claims to the contrary were based on the use of inadequate techniques (*J. Neurophysiol.,* **11**: 125, 1948). Having met the challenge, the theory emerged from these discussions stronger than before (*Johns Hopkins Bull.* in press).

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[1] Biochemical Institute, University of Stockholm.

The ion permeability of the giant axon of squid. M. A. Rothenberg.

The difference in concentration of ions between the inside and the outside of nerve fibers has long been assumed to be of importance in conduction (Ostwald, Bernstein, and many others). Most of the evidence suggesting ion movements during the passage of the impulse across the active membrane has been, however, of an indirect nature (see Hodgkin, *J. Physiol.*, **106**: 341, 1947). The exchange of ions across the nerve membrane of the giant axon of squid has now been measured by the direct determination of radioactive Na^{24} and K^{42} in the axoplasm of these fibers. In all cases, the outer environment was artificial sea water prepared according to Pantin (*J. Exp. Biol.*, 11: 11, 1934), and no alteration in the concentration of ion species was made.

The results appear to indicate that during rest the ions inside the fiber may be in dynamic equilibrium with the same ion species outside. Thus, within 20–30 minutes all of the Na^{23} inside the fiber (based on figures of Steinbach and Spiegelman, *J. Cell. and Comp. Physiol.*, **22**: 187, 1943) exchanges for Na^{24} in the sea water. In the case of K^{42}, only about 10 per cent of the total inside will exchange within the same period of time. A twofold increase in the K^{42} penetration rate could be obtained with a two-fold increase in the K^{42} concentration in the sea water.

Attempts were made at determining the temperature coefficients for the Na^{24} and K^{42} exchanges across the membrane. A ten degree difference in temperature of the bathing fluid (12° and 22° C. resp.) showed no marked alteration in the rates of exchange. This supports the assumption that at rest there are no important chemical reactions involved in this exchange other than a Donnan equilibrium.

Electrical activity of the nerve increased markedly the rate of Na^{24} penetration. This same overall effect could be obtained by the addition of anticholinesterases to the sea water. For example, the addition of diisopropylfluorophosphate increased the Na^{24} penetration by about 50 per cent, and decreased the K^{42} penetration by about 35 per cent. This suggests an increase in membrane permeability and may, when studied more closely, throw some light on the immediate function of acetylcholine and cholinesterase in nervous conduction.

Cocaine, unlike the anticholinesterases, had a very small effect on the membrane permeability.

July 27

The extraction of purified squid "visual purple." A. F. Bliss.

The photochemistry of the squid retina has been studied by methods which have been successfully applied to vertebrate retinas. The common vertebrate visual pigment, rhodopsin, is replaced in the squid by a photostable homolog, cephalopsin. This pigment has been extracted in a purified state by a combination of Saito's and Lythgoe's methods for rhodopsin. Squid retinas are homogenized with 40 per cent sucrose and centrifuged at a high speed. This treatment separates the heavier melanin from the lighter red segments which contain the visual pigment. The red rod layer floats to the top and after separation is hardened with buffer at pH 4.5. It is then extracted with a mild detergent, aqueous digitonin. A clear red extract is obtained, whose absorption spectrum closely resembles that of rhodopsin. The maximum due to cephalopsin is at about 495 $m\mu$ in the blue green, while that due to rhodopsin is at 502 $m\mu$. Cephalopsin becomes photosensitive in the presence of five per cent formalin and bleaches almost completely when exposed to light of a 100-watt lamp at a distance of one foot for 20 minutes. It is also bleached by strong acid and base in the dark. The bleaching products are indicator yellow and retinene, precisely as obtained from bleached rhodopsin. Cephalopsin apparently does not bleach *in vivo*. The increased retinene released from illuminated retinas shaken in petrol ether reported by Wald is probably a secondary physical effect due to pigment migration which increases the effective diffusion surface of the illuminated retina. Pigment migration fails in aerated retinas kept one hour after excision in complete darkness whereas Therman has shown that such retinas actually are more sensitive to light than freshly excised retinas. Retinas prepared according to Therman's procedure exhibited a light: dark retinene ratio of 1.02 ± 0.8, which does not differ significantly from unity. A detailed account of the purification of cephalopsin will appear in the *Journal of Biological Chemistry*.

Biochemical and histochemical observations on the sexual dimorphism of mouse submaxillary glands. L. C. JUNQUEIRA, A. FAJER, M. RABINOVITCH AND L. FRANKENTAHL.

Protease and amylase activity is demonstrated in extracts of mouse submaxillary glands. The protease content varies sexually but the amylase content does not. In castrated adult mice steroid sexual hormones influence the protease content of the glands. Correlation of biochemical and histological data locates the site of protease production in the tubular portions of these glands, and amylase synthesis in the acinar regions. Evidence is presented for location of ribonucleoproteins in the basal regions of acinar and tubular cells, and for variations in its content with sexual variations in the tubular cells. The secretory granules of the tubular cells appear to be of protein nature, as they give a positive reaction for phenolic amino acids and for sulfhydrilated proteins. "Acid" phosphatase is evidenced in the apical portions of the tubular cells, and shows histochemical and biochemical sexual variations. "Alkaline" phosphatase is distributed more diffusely throughout the gland. Our data suggest, but do not establish, sexual variation of the activity of this enzyme. Mucoproteins are present in the cytoplasm of the amylase producing acinar cells.

pH *estimation in reconstituting pieces of Tubularia stems.* JAMES A. MILLER, JR.[1]

Phenol red, chlor phenol red, brom thymol blue, brom cresol purple and brom cresol green were injected into the coelenteron of Tubularia with the aid of a Chambers micromanipulator. Of these, phenol red proved the most satisfactory both because of low toxicity and appropriate range.

The pH of uninjured stems ranged between 7.8 and 8.0. Cutting, crushing, inserting a pipette or even bending the stem sharply caused the release of an acid of injury the concentration of which depended upon the degree of injury. Recovery of normal pH required one to fifteen minutes, depending also on extent of injury.

Reconstituting stems released acid metabolites which maintained the coelenteric fluid at 0.2 to 0.4 pH below that of the uninjured stem. The first morphological indications of hydranth reconstitution were accompanied by regions of increased acidity corresponding to the two rows of tentacles. Once formed, the tentacles remained acid (pH 6.8 to 7.0) as also did the ring of perisarc-secreting tissue just proximal to the hydranth. The pH of other parts could be increased by increasing the availability of oxygen. Stolon formation and growth was not accompanied by observable changes in pH.

When reconstitution was blocked by placing stems in glass tubes, acidity was increased in all parts equally. Ligatured stems, on the other hand, showed the normal pH and in some cases developed regions of increased acidity at the ends indicating partial activation. One should therefore guard against considering that all ligatured stems are under truly basal conditions.

These experiments demonstrate that acid metabolites are produced in the ectoderm and endoderm of Tubularia and are liberated into the coelenteron. If prevented from escaping they increase the acidity to a point which has been found to inhibit reconstitution when externally applied.

The genetic block to free oviposition in the chalcidoid wasp Melittobia sp.—C. P. W. WHITING AND BERTINA M. BLAUCH.

Melittobia females normally fail to oviposit freely unless mated. The very rare exceptions reported ("layers") produce large progenies (100 to 300) of haploid sons. From a stock derived from a layer, 100 virgin females were isolated. The test showed 15 layers and 85 nonlayers, the latter producing not more than four sons each. Sons of the layers were crossed with their aunts. Of 150 daughters tested, 51 were layers. The trait is clear-cut with no intergrades. The genetic basis, however, is complex. Selection, inbreeding and crossing the descendents for seven generations showed layer females per fraternity varying from 0 to 100%. From sibling matings in one line, three fraternities totalled 6 layers, 355 non-layers (1.66% layers), three fraternities totalled 84 layers, 326 non-layers (20.49%), one fraternity totalled 73 layers, 77 non-layers (48.66%). It was noted that layer females have a shorter life than non-layers and that there is close association of the trait with sterility and short life of their sisters. One layer when self-crossed (mated to a haploid son) produced 27 layers, 31 non-layers and 29 dying sterile after ten days (33.33% sterile). Another produced 22 layers, 4 non-layers and 20 dying sterile (43.48% sterile). Four produced 46 layers, no non-layers and 93 dying sterile (66.91% sterile). One self-crossed layer produced 6 layers, 240 non-layers (sterile not recorded). The trait is evidently not a recessive. Of these 240 non-layers, 15 were self-crossed. Grouped according to increasing percentage of layers and arranged as layers/non-layers/dying sterile, there were two fraternities, 3/73/12, with 13.64% dying sterile, five, 25/189/34, with 13.71%, one, 7/29/14, with 28.00%, five, 58/125/72, with 28.23%, and two, 34/37/28, with 28.28%. It is indicated that more than two factors may be involved with complementary effect and that the group dying sterile is genetically related to the layers.

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[1] Emory University.

August 3

Inhibition of sea urchin egg cleavage by a series of substituted carbamates. Ivor Cornman.[1]

Urethane (ethyl carbamate) is both carcinogenic, inducing lung tumors in rats and mice, and carcinostatic, diminishing tumor growth in rats and mice and lowering wbc count in murine and human leukemia. To correlate these properties with efficiency as a mitotic poison, eggs of *Lytechinus* and *Tripneustes* were exposed to a series of carbamates with methyl, ethyl, propyl, butyl, amyl and phenyl replacing the ethyl on the carboxyl or hydrogens on the amino end of urethane. These were supplied by Dr. C. D. Larsen of the National Cancer Inst. Exposure was begun 10 minutes after fertilization. Delay and blocking of cleavage were compared quantitatively. In general, effectiveness in blocking cleavage increased with increase in M.W. up to 8–10 carbon atoms. Isopropyl N-phenyl carbamate, Ethyl N,N-di-*n*-butyl carbamate and ethyl N-phenyl carbamate were the most efficient, blocking cleavage at 0.5–0.6 m Molar, while urethane required 56 mM/L. A cyclic configuration was the most effective, then straight-chain, branched-chain (*iso*), and divided groups (N,N-dimethyl, etc.), in decreasing order of activity. This parallels the narcotic activity of carbamates, but is entirely divergent from efficiency of pulmonary adenoma induction or of tumor suppression. For both of these, urethane is reported as the most active of the carbamates tested. These remarkable influences on the neoplastic process appear not to devolve in any direct way from destruction of mitosis.

A nuclear precursor to ribo- and desoxyribonucleic acids. A. Marshak.[2]

Rats were given P^{32} Na_2HPO_4 intravenously and nuclei isolated from the liver three hours later. Incubation of the nuclei at 37° C. with ribonuclease, desoxyribonuclease and with no added enzyme showed that 90 per cent of the nuclear P^{32} was in nucleic and the remainder in lipid and acid soluble fractions. Very little or none of the P^{32} was in DNA in nuclei from normal liver (no mitosis) and also in nuclei from regenerating liver (rapid mitosis). Incubation at 0°–2° C. releases no nucleic acid from the nuclei, although at 37°, 80 per cent is removed indicating the presence of an enzyme capable of splitting nucleoprotein or nucleic acid. The digestion products are not dialyzable and therefore are nucleic acid and not nucleotide. The native enzyme thus differs from ribonuclease which splits off nucleotide. The specific activity of the digestion products is 13 times as great as that of the RNA of the cytoplasm. The material extracted from the nuclei by the method for extracting RNA has a specific activity almost as high as that of the nuclei. This fraction may therefore be identical with the P^{32}-containing nuclear substrate but both are very different in behavior from the RNA of the cytoplasm. Extraction of the nuclei for 24 hours with 1 M NaCl removes only 20 per cent of the P^{32} and the P^{32}-containing material so extracted is not precipitated on dilution to 0.14 M NaCl. The P^{32}-containing nuclear material thus differs in solubility from desoxyribonucleoprotein. On the basis of the orcinol and diphenylamine reactions the nuclear auto-digestion products contain "RNA" and an amount of "DNA" less than 1/10 of the "RNA." Analyses of uracil and thymine con-

[1] Sloan-Kettering Inst. for Cancer Research.

[2] New York University College of Medicine. Tuberculosis Control Division, United States Public Health Service.

tent by preparing the p-iodophenylsulfanile derivatives with radioactive iodine show a thymine content less than 1/10 that of the uracil. In saline, desoxyribonuclease increases the rate of splitting of the P^{32}-containing substrate but in carbonate buffer at pH 7.0 it reduces the rate, suggesting binding of substrate without splitting it. The nucleic acid in question thus appears to have some properties in common with both DNA and RNA. In both mitotic and non-mitotic nuclei the P^{32} appears first in this nucleic acid; later, in mitotic cells, the P^{32} is accumulated in DNA, while in non-mitotic cells it passes into RNA of the cytoplasm, the P^{32} always being associated with nitrogenous base and sugar. Since it contributes to the formation of DNA and RNA and also since it has properties in common with each this nucleic acid is considered to be their precursor.

These findings indicate that plasmagenes and other cytoplasmic constituents containing nucleic acid cannot be independent of nuclear activity. They also predict that cells may be found which contain no DNA. Strains of bacteria with no desoxyribose and with no thymine have been reported.

The effect of "stabilizing" and "unstabilizing" agents in relation to the metabolic mechanism supporting the resting potential of nerve.[1] ABRAHAM M. SHANES.[2]

Certain agents which block conduction (e.g. calcium, cocaine, procaine) are known to enhance the polarization of nerve fibers; others, which increase excitability (e.g. calcium precipitants, veratrine), have been observed to cause depolarization. The former have been designated as "stabilizing," the latter as "unstabilizing."

It is now possible to characterize these two groups of substances by another effect, viz., their ability to modify the rate with which anoxia leads to depolarization. Thus, cocaine and procaine, in concentrations far below those which affect respiration (e.g. 0.001%), delay the decline of the resting potential in frog nerve during oxygen lack. Associated with this is a delay in the development of inexcitability. Spider crab (*Libinia emarginata*) leg nerves also are rendered less sensitive to anoxia by procaine, but the concentration required (ca. 0.05%) causes depolarization in oxygen.

Veratrine, like calcium precipitants, in a concentration which causes negligible depolarization in oxygen (1:200,000 for frog sciatic nerve, 1:2 million for crab nerve), speeds the depolarization process during anoxia and, when depolarization has not been excessive, increases the potential rise upon return to oxygen.

The exactly antagonistic effects of the stabilizing and unstabilizing compounds with respect to excitability, resting potential, and anoxia sensitivity suggest the involvement of a single basic locus. An explanation in terms of potassium permeability is in keeping with the available literature on the effects of these substances on potassium leakage and impedance; this is supported further by (a) the reduced depolarizing action of KCl on frog muscles treated with cocaine and (b) the ability of cocaine and procaine (0.2%) to slow the swelling of muscles in a Ringer in which some of the sodium has been replaced by potassium.

AUGUST 10

The relative rate of penetration of the lower fatty acids into beef red cells. JAMES W. GREEN.

Since the work of Overton the lipid solubility theory has been widely held to account for the penetration of lipid soluble compounds into cells. This theory may be tested by a study of the penetration rates of the lower fatty acids into mammalian erythrocytes. When a weak acid penetrates a mammalian red cell the oxyhemoglobin within the cell dissociates to a new equilibrium, the level of which appears to depend upon the amount and dissociation constant of the acid which entered the cell and the oxygen tension of the environment. Taking advantage of this dissociation of oxyhemoglobin, a modification of the Hartridge-Roughton rapid mixing technique was used to measure spectrophotometrically the rate at which the lower, saturated, monocarboxylic acids penetrated beef red cells. The method is essentially chemical and does not depend upon volume changes of the cell. Dilute unbuffered red cell suspensions were mixed with dilute acid solutions made up in 1 per cent NaCl such that the pH of the final mixture was maintained in the range 4.2.

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[1] This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service, and from the American Philosophical Society.

[2] Georgetown University School of Medicine.

The relative order of increasing rate of penetration of the acids studied for beef cells was found to be: formic, acetic, propionic, caprylic, heptylic, caproic, butyric, valeric. However, the rates of penetration of caproic, valeric and butyric acids could not be separated statistically. The actual time to 50 per cent penetration was calculated and found to vary from 0.138 seconds for valeric acid to 5.04 seconds for formic acid. From formic through caproic acids the relative rates of penetration are in agreement with results obtained by other methods and are in accord with the Overton theory. Heptylic and caprylic acids, although more lipid soluble than lower homologues, penetrate beef red cells more slowly than acids of smaller molecular volume. For this reason it is suggested that these two acids are limited in their penetration by reason of their larger molecular volumes.

Hemolysis studies were made with these acids, using the Parpart Densimeter. The hemolysis curves obtained, taken as a measure of penetration of these acids, did not support the findings using the spectrophotometric technique. It is thought that the discrepancies were owing to the inability of the hemolytic technique to distinguish between osmotic and lytic hemolysis.

Osmotic hemolysis in hypertonic solutions.[1] F. R. Hunter.[2]

It has previously been noted in this laboratory that chicken erythrocytes standing for 12–24 hours in heparinized plasma at 37° C. become altered. Using a photoelectric apparatus to measure volume changes of these cells when they are placed in a hypertonic solution consisting of 0.3 M glycerol in Ringer Locke, a greater deflection of the galvanometer is noted when older cells (those which have stood at 37° C. for several hours) are used, as compared with new cells (those in freshly drawn blood). The apparent volumes, as measured photoelectrically, of both new and old cells in Ringer Locke, 2X Ringer Locke and 2X Ringer Locke to which an equal volume of water has subsequently been added are what would be predicted on the basis of the swelling curves. Spectrophotometric measurements of the amount of hemolysis in these various solutions show that as the cells stand in heparinized plasma at 37° C. there is an increase in their "fragility" as indicated by a large amount (up to 46 per cent) of hemolysis when old cells shrink and swell, the equilibrium medium being Ringer Locke. This hemolysis is noted whether the volume changes involve the penetration of the glycerol molecule or whether the cells shrink in 2X Ringer Locke and then swell again as a consequence of dilution of the medium with water. Hematocrit measurements show an increase in volume (5–20 per cent) as the cells stand in heparinized plasma. These cells also lose potassium and gain sodium. These experiments emphasize further the extreme sensitiveness of erythrocytes to their environment.

Hippuric acid excretion in anxiety states. Harold Persky.

The liver function of human subjects was determined by a variety of standard tests employed by the clinician. Only the sodium benzoate tolerance test (intravenous) was significantly altered in patients suffering with anxiety states. Controls were normal persons and other psychoneurotics hospitalized under identical conditions to the anxiety group but showing little visible anxiety.

The experimental and control subjects were physically healthy adults between the ages of 16 and 58. They had no clinical indications of liver disease nor any previous history of liver disease. They were in good nutritional status as judged by clinical examination and by a three day nitrogen balance study performed just before the liver function tests. The subjects had good kidney function as indicated by a normal serum urea level and in some instances also, a normal urea clearance.

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[1] This work was supported by grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service and the Faculty Research Fund, The University of Oklahoma.

[2] Department of Zoological Sciences, The University of Oklahoma, Norman.

The degree of anxiety was assessed by a variety of clinical and psychological tests with general agreement among them qualitatively for each subject. For quantitative correlation, a new method of scoring the Rorschach test for anxiety was employed.

The anxiety group excreted an abnormally high amount of hippuric acid after i.v. injection of sodium benzoate. This effect was not observed in either of the two control groups. The excessive amount of hippuric acid in some instances was even greater than the theoretical yield of hippuric acid obtainable from the injected benzoate. The excretion of hippuric acid was directly related to the degree of anxiety for the anxiety state group. The relation is significant statistically. There is no correlation for the control groups.

Following psychotherapy, the hippuric acid is significantly decreased when the anxiety is decreased. The psychotherapeutic techniques used were: electric shock, insulin shock and/or psychotherapy.

The elevated hippuric acid excretion in anxiety states is due to an elevated endogenous hippuric acid production in anxiety states over the control groups. This endogenous hippuric acid exceeds that of the control groups from two to ten times. The absolute amount is adequate to explain the supernormal values obtained when sodium benzoate is administered.

The endogenous hippuric acid probably is derived from phenylalanine metabolism. It is postulated that the excessive hippuric acid obtained in anxiety states is due to diversion of phenylalanine employed for adrenaline synthesis resulting in an autonomic inbalance.

Biological specificity and protein structure. DOROTHY WRINCH.[1]

Studies on the relation between biological function and form are already so far advanced that it is universally recognized that biological specificities belong to the angstrom world. There is no longer any doubt that the fundamental questions involved can be formulated—and subsequently studied and finally elucidated—only by recourse to the nature of the atomic patterns and electron density distributions. The studies of the crystal forms of about 170 different hemoglobins (Reichert and Brown, *The crystalline hemoglobins,* Washington, D. C., 1909) which have lain uninterpreted for nearly 40 years prove to yield material of the first importance for an understanding of biological specificities (Wrinch, *Am. Mineral.* in the press). The prevalence of twins and intergrowths among these protein crystals and their pseudosymmetries in many cases are found to relate them closely to a number of minerals for which the atomic patterns are already established. Attention is called to the strong case which can be made out for an isostructural relation between the oxyhemoglobin of *Necturus maculatus* and Staurolite ($H_2FeAl_4Si_2O_{12}$), between the oxyhemoglobin of *Cavite cutleri* and Tetrahedrite (Cu_3SbS_3). To interpret these striking facts, which stem from the forms of the crystals and the cube and double cube nature of the twins, trillings and other intergrowths, it is necessary to find a generalization of the structural essence of these minerals in terms applicable to proteins. Crystallographic studies have demonstrated how (e.g.) the carbon *atoms* in diamond may be replaced by *molecules,* in Sernarmontite or Arsenolite or the 29-hydrate of phosphotungstic acid (Wrinch, *Phil. Mag.,* **38**: 373, 1947: Wallerstein Communications in the press). By means of a basic principle of successive generalization, these molecules may, in turn, be replaced in appropriate circumstances by regular crystal-like arrays of molecules to give particles, crystals and intergrowths in general. It appears, therefore, that the major cubic theme in Staurolite and Tetrahedrite should be interpreted for the hemoglobins as cubic skeletons of interlocked α-levo amino acid backbones. The accompanying minor theme, which may or may not be cubic, is then seen to mean the R-substituents on the skeleton—the "fluff" or "spines" on the surfaces of protein molecules—together with the accompanying foreign molecules or ions in the crystal.

The postulate of a cubic arrangement of interlocked backbones in protein skeletons points the way to orderly arrangements of molecules into particles. Particles in the cubic and double cube systems, sometimes with disturbing non-cubic "fluff," containing 2, 3, 4, 5, 6, 7, 8, 12, 16, . . . molecules are suggested for consideration in the cases of the hemoglobins, insulin, horse serum albumin, ribonuclease, etc.

The picture suggested by the hemoglobin data thus includes (a) a general aspect embodying the protein essence in a cubic skeleton for the individual molecules, a number of molecules (possibly 12 with 48 residues apiece in many different hemoglobins) representing each individual particle and (b) a particular aspect embodying the protein specificity which resides in (b1) the pattern and associational complexes of the fluff or spines emerging from the skeletal surfaces and in (b2) the resulting characteristic differences in molecular patterns. In the forms of individual crystals and in the forms of their intergrowths in general lies a direct approach to the biological specificities of different hemoglobins *at the angstrom level.*

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[1] Smith College.

(This work is supported by the Office of Naval Research under contract N8onr-579.)

AUGUST 17

The incorporation of carbon dioxide into organic linkage by retina. R. K. CRANE, E. G. BALL, AND A. K. SOLÓMON.

The incorporation of carbon dioxide into organic linkage first demonstrated in bacteria by Wood and Werkman (*Biochem. J.,* **32**: 1262, 1938) and in pigeon liver by Evans and Slotin (*J. Biol. Chem.,* **136**: 301, 1940) has since been shown to proceed by the beta carboxylation of pyruvic acid (Wood-Werkman Reaction). The incorporation first observed in the whole rat by Hastings et al. (*J. Biol. Chem.,* **140**: 171, 1941) has been assumed to occur by the same pathway. The present investigation confirms and extends the previous observations on avian tissues and provides a more direct demonstration of this reaction in the mammal.

Tissues were incubated with pyruvate at 37° C. in a closed vessel containing carbon dioxide and bicarbonate labelled with carbon fourteen. Excess pyruvate was isolated as the 2,4-dinitro phenylhydrazone. The hydrazone was recrystallized and its radioactivity determined. In a survey of various tissues under standardized conditions the following rates of CO_2 incorporation were found: pigeon liver, 37; duck retina, 17; ox retina, 11; rat liver, 4; rat kidney, 3; pigeon heart, 0.6; rat heart, 0.5; and pigeon breast muscle, 0.3. Retinas were used whole, all others were sliced. The extensive damage on slicing may account for the low rate in muscular tissues.

Since ox retina was the most active mammalian tissue, it was studied further. The rate of incorporation by this tissue was found essentially constant for at least four hours. Varying the CO_2-bicarbonate buffer system showed that optimum conditions exist when the bicarbonate ion does not exceed 20 millimols per liter. Increases above this decrease the rate whether the pH is or is not held constant by increasing the CO_2 tension. Within the range studied, pH (7.1–7.7) and CO_2 tension (5–20 per cent) appear to have little influence. Anaerobiosis reduced the incorporation rate by 70 per cent with no additional effect on the addition of 0.01 molar iodoacetate. The same concentration of iodoacetate added aerobically caused a 60 per cent reduction. Ammonium ion (0.01 molar) produced a 70 per cent reduction under aerobic conditions.

Ultrastructure of the nerve axon. E. DEROBERTIS (no abstract submitted).

Mechanisms of interaction of inhibitions with plasma cholinesterase. A. GOLDSTEIN (cf. Lalor Reports).

The synthesis of nucleoproteins in developing Arbacia studied with the aid of P^{32}. CLAUDE A. VILLEE, M. LOWENS, M. GORDON, E. LEONARD, A. RICH (cf. Lalor Reports).

GENERAL SCIENTIFIC MEETINGS

AUGUST 24

Enzyme localization in the giant nerve fiber of the squid. BENJAMIN LIBET (cf. Lalor Reports).

Choline esterase choline acetylase ratio in invertebrate tissues. HAROLD PERSKY (cf. Lalor Reports).

Non-integrative synapses. THEODORE H. BULLOCK.[1]

Synapses have been defined as valves which must change their conditions of openness from time to time in ordinary functions, or remain but partially open. That is they must integrate (make each presynaptic impulse count for more or less than one in eliciting an outgoing discharge Prosser). Until recently this concept could not be denied on grounds of known neuroneural junctions. There are now several synapses found among invertebrates which behave somewhat like the classical vertebrate neuromuscular junction: they apparently act normally as simple relays, passing every impulse that arrives in a 1 : 1 manner.

The giant synapse in the stellate ganglion of the squid may be such a case. Except in advanced stages of fatigue no summation, spatial or temporal, occurs, facilitation and after discharge are absent, though it is still possible that different paths as yet undetected physiologically may alter this. The junctions between central giants and motor giants in the ventral cord of the crayfish act in this simple relay fashion (Wiersma). There are evidently commissural synapses between the two lateral giants in the same animal which normally conduct in a 1 : 1 and, furthermore, an unpolarized manner. Finally, the septal junctions which recur segmentally in these same crayfish laterals and in the earthworm giant fibers act likewise until severely fatigued. These septa seem likely, on the basis of accumulating evidence (unpublished), to be real functional as well as anatomic barriers and therefore synapses on a less arbitrary definition.

What biologic meaning can such relay junctions have? Unless new afferents are formed must they be regarded as present only for the occasions when fatigue is advanced, or as trophic boundaries, embryonic rests or the meeting of incompatible protoplasms (these same species have demonstrated that neuronal fusion can occur in suitable situations)? Are they primitive or specialized junctions? Whatever their interpretation it seems necessary in the face of new evidence to broaden our conception of the synapse.

Phosphagen in annelids (Polychaeta). ERNEST BALDWIN AND WARREN H. YUDKIN (cf. Lalor Reports).

Crustacyanin, the blue carotenoid-protein of the lobster shell. GEORGE WALD, NEAL NATHANSON, WILLIAM P. JENCKS AND ELIZABETH TARR.[2]

It has been suggested that the striking change in color which the lobster shell undergoes on boiling is caused by the splitting of the red carotenoid astaxanthin from a blue complex with protein (Kuhn and Sörensen, *Ber. deutsch. chem. Gesel.*, 71: 1879, 1938). Kuhn and coworkers, however, were unable to extract such a complex from the shell, in which they believed it to be held by calcium deposits.

We have extracted a deep blue astaxanthin-protein from the lobster shell with dilute citric acid. This substance, called crustacyanin (i.e., shell blue), possesses a broad absorption band maximal at about 625 mμ. It is precipitated from solution by 40 per cent saturation with ammonium sulfate, and dissolves in distilled water. Its isoelectric point lies below pH 4.5. On denaturation by heating at 100° C. or treatment with mineral acids, alcohols or acetone in the warm, the blue solutions turn orange-red, the absorption maximum moving to about 460 mμ. The denatured solutions, on dilution with acetone or alcohol, yield all their color to neutral fat solvents in the form of astaxanthin.

If crustacyanin is warmed to 60° C. in m/10 veronal buffer, pH 7, it exhibits a type of reversible denaturation. The color goes from blue to purple, the absorption maximum from 625 mμ to about 530 mμ. On cooling, the color returns to blue, the absorption band to its previous position.

Crustacyanin preparations frequently display a narrow absorption peak at about 412 mμ.

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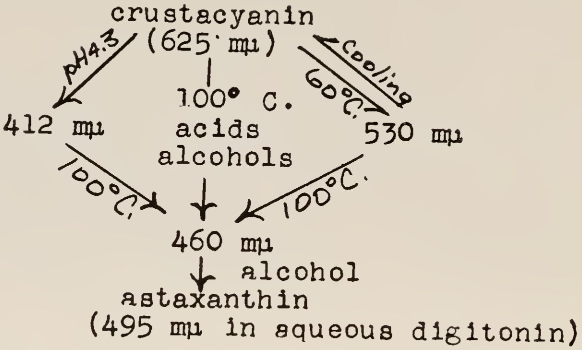
[1] University of California, Los Angeles.

[2] This investigation was supported in part by the Medical Sciences Division of the Office of Naval Research.

This is very variable in height and in our best preparations is completely absent. Some very close relation exists between the 625 and the 412 mμ absorptions. When crustacyanin is brought to pH about 4.3 the 625 mμ band falls while the 412 mμ peak rises proportionately. The final product possesses the 412 mμ band alone. If this is heated it goes over to the orange-red color and 460 mμ absorption of heat-denatured crustacyanin.

These relations are summarized in the following diagram:



The activity and distribution of desoxyribonuclease and phosphatases in the early development of Arbacia punctulata.[1] DANIEL MAZIA, GERTRUDE BLUMENTHAL, AND ELEANORE BENSON.

The original aim of this investigation was to find enzymes that were associated with the nucleus and to follow their behavior through nuclear division. Desoxyribonuclease (DNase) seemed a logical choice because its substrate is restricted to the nucleus. There has also been evidence of nuclear localization of phosphatase (Pase). DNase was determined by the method of Barton and Mazia, involving separation of the high polymer DNA by precipitation with protamine. In the Pase measurements, glycerophosphate was used as substrate and inorganic P was determined.

Nucleated and enucleated fragments of unfertilized eggs were obtained by the Harvey method. There was no clear indication of sharp nuclear localization of acid Pase (pH 5) or alkaline Pase (pH 9). Desoxyribonuclease was not restricted to the nucleated fragments. These generally had a higher activity per fragment, but not per unit volume. The sum of the activities of the fragments was 30–40 per cent higher than that of the whole eggs. It is indicated, therefore, that most of the DNase activity of the unfertilized egg is in the cytoplasm, though DNA has not been found there in measurable amounts.

The DNase activity was followed through developmental stages to the 40 hour pluteus. Total activity and the activity of the "soluble" fraction (not sedimentable at 20,000 g.) were measured. Total activity did not increase significantly during development. The non-sedimentable fraction declined steadily, reaching at 40 hours a value 10–20 per cent of the total activity. The enzyme seems to be fixed on structural components of the cell, possibly nuclei, as development proceeds.

In the unfertilized egg the acid Pase predominates. The activity is 3–5 times that of alkaline Pase. During development, acid Pase remains constant. Alkaline Pase remains constant until just before gastrulation, then rises steadily to a value 10 or more times that of the unfertilized egg.

These findings make no contribution to the original question of the behavior of nuclear enzymes in cell division but do show the existence of two different patterns of development of enzyme systems in morphogenesis. One is the formation of new enzyme (alkaline Pase) in the course of development. The other is the storage of adequate amounts of enzymes (DNase) during oogenesis and the subsequent structural differentiation of the enzyme as morphogenesis proceeds.

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[1] Work supported by grants from NRC Committee on Growth, acting for the American Cancer Society, and from University of Missouri Research Council.

The effects of nitrogen mustards on cleavage and development of Arbacia eggs. John O. Hutchens and Betty Podolsky.

Effects of tris(β-chloroethyl) amine; bis(β-chloroethyl), methyl amine; bis(β-chloroethyl), isopropyl amine; n-butyl, bis(β-chloroethyl) amine; benzyl, bis(β-chloroethyl) amine; bis-(β-chloroethyl), β-methoxyethyl amine; and bis(β-chloroethyl) furfuryl amine on early cleavage and developmental stages attained at 12, 24, and 48 hours were studied using fertilized eggs of *Arbacia punctulata.* Amine hydrochlorides dissolved in 0.52 M NaCl were added to suspensions of eggs in sea water 20 minutes after fertilization. One hour after exposure the eggs were washed twice in sea water. Temperature was 22° C.

Nitrogen mustards in non-cytolyzing concentrations slow, rather than completely block, division. At 3 hours only about 50 per cent reduction in number of cleavages can be effected without cytolysis. Concentrations of various compounds reducing cleavage to 75 per cent of the control value at 3 hours were therefore compared. Tris(β-chloroethyl) amine is most effective, a dose of 2.8×10^{-5} M being required. The various bis(β-chloroethyl) amines are all about one-tenth as effective, doses of $2\text{–}3 \times 10^{-4}$ M being required.

Cleavage in the presence of nitrogen mustards was frequently irregular, some blastomeres dividing more slowly than others. If cytolysis did not occur, apparently normal ciliated blastulae eventually developed. Gastrulation was blocked, however, even at concentrations which only slightly inhibited early cleavage. 10^{-5} M tris(β-chloroethyl) amine or 10^{-4} M solutions of the other six compounds prevented normal pluteus development at 24 hours. That micromere formation and setting aside of proper proportions of animal and vegetal cells is not involved is indicated by the fact that ciliated blastulae (ca. 8 hrs. development) exposed to these same concentrations were inhibited to about the same extent at 24 hours.

Respiration of the fertilized egg is not inhibited significantly by these concentrations of nitrogen mustards. Respiration of treated embryos, in fact, increased with time, corresponding to the developmental stages attained.

The effects of pressure on the insemination reactions of Arbacia eggs. Douglas Marsland.[1]

A syringe placed inside the microscope-pressure chamber permits sperm to be ejected upon the eggs exactly 10 seconds before pressures up to 15,000 lbs./in.^{2} are applied. At higher pressures (6000 lbs. and above) the egg gives no visible reaction to the sperm. Numerous active sperm may come into contact directly with the egg surface, but the fertilization membrane does not lift and the hyaloplasma layer does not appear so long as the pressure is maintained. But as soon as the pressure is released, the fertilization membranes and the hyaloplasma layers begin to appear; and subsequently the eggs develop normally, at least to the free-swimming stage, provided the period of high pressure does not exceed about 15 minutes. However, the lifting of the fertilization membranes in the pressure treated eggs is slower and less complete than in untreated eggs; and for pressures exceeding 8000 lbs., no fertilization membranes can be distinguished on most of the eggs, although the hyaloplasma layer becomes distinctly visible after the pressure is released, and the cleavages go ahead on schedule. Also pressures above 6000 lbs./in.^{2} must reversibly block the penetration of the sperm into the egg, since control eggs fertilized at the instant when the pressure is released undergo the first cleavages in exact synchrony with eggs which had been fertilized earlier and then immediately exposed to a 15 minute period of compression.

Below 6000 lbs./in.^{2} the pressure inhibition of the insemination reactions is less complete. At 4000 lbs. the sperm can penetrate the egg surface during the compression period and the hyaloplasma layer can form, although the lifting of the fertilization membrane is suppressed in almost all the eggs until the pressure is released. And while a pressure of 2000 lbs. is maintained, the only observable effect is a delay in the penetration of the sperm, the formation of the hyaline layer, and the lifting of the fertilization membrane.

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[1] Professor of Biology, Washington Square College of Arts and Science, New York University.

Lactones as mitotic poisons, tested on sea urchin eggs. IVOR CORNMAN.

The lactone ring is prominent in many physiologically active compounds produced by plants, unsaturated lactones being especially active. Experiments with the eggs of *Lytechinus variegatus,* beginning exposure 10 minutes after fertilization, show that effectiveness in blocking cleavage increases 1000-fold with shifts in the position and number of the double bonds. Alpha angelica lactone (3-pentene-1,4-olide) slows the second cleavage 4 per cent at 0.11 mMolar, and blocks eggs in the 2-cell stage at 1.13 mMolar. Moving the double bond to carbons 2 and 3 (β angelica) decreases the activity by a factor of 10. However, adding a double bond (2,4-pentadiene-1,4-olide: protoanemonin) gives a compound which blocks all cleavage at 0.01 mMolar. Coupling two of these molecules at carbons 4 and 5, thereby destroying the second unsaturated linkage (anemonin) reduces the activity: blocking at 0.52 mMolar. A comparable decline in potency comes with moving the 4,5 double bond farther out a side chain (γ-propenyl-γ-crotonolactone) or hydrolysing it (2-pentene-4-hydroxy-1,4-olide).

At these concentrations which block cleavage, there is first a development of the achromatic figure. It is then inactivated, as evidenced by the incomplete separation of anaphase chromosomes in α-angelica lactone. Flakes of hyaline material in eggs treated with anemonin and protoanemonin suggest dispersal of the achromatic figure without its complete resorption. Studies have already been made of the effects of lactones on plant mitoses (Erickson and Rosen, *Science,* in press).

Penetration and effects of low temperature and cyanide on penetration of radioactive potassium into the eggs of Strongylocentrotus purpuratus and Arbacia punctulata. E. L. CHAMBERS, W. WHITE, NYLAN JEUNG AND S. C. BROOKS.[1]

Radioactive potassium was added to 0.2 per cent suspension of (1) unfertilized, and (2) fertilized eggs of *S. purpuratus* at 15° C., giving a $[K^{42}]$ of .015 μc/ml. Samples were removed at intervals for determinations of radioactivity, and for chemical analyses (cf. method, abstract presented by title, Penetration of Radioactive Phosphate, etc.).

In the unfertilized egg the exchange rate is gradual. The total quantity of K^{42} entering reaches a maximum in 15 hrs. At this time the specific activity of the eggs: $\frac{[K^{42}\text{ inside}]}{[K^{39}\text{ inside}]}$ equals 20 per cent that of the suspension fluid: $\frac{[K^{42}\text{ outside}]}{[K^{39}\text{ outside}]}$.

In the fertilized egg the exchange rate increases immediately, the maximum being reached in 30 minutes. At the time of first cleavage (110 minutes) the specific activity of the eggs had reached 50 per cent that of the suspension fluid. During this period no increase in content of K occurred. A maximum is reached in 15 hrs. when the specific activity approaches about 85–90 per cent that of the suspension fluid. During the first two hours of development (one cell stage) the total exchange rate of K in the fertilized egg was found to be 7–13 times that of the unfertilized.

The tentative assumption is that the total quantity of readily exchangeable K approximates 20 per cent in the unfertilized egg and 85 per cent in the fertilized. By making the calculation on this assumption the rate of approach to equilibrium of the freely diffusible K is only 2–3 times more rapid in the fertilized egg. A similar relation was found in the eggs of Arbacia.

Exposure of the fertilized *purpuratus* eggs for a short period to low temperature, and to cyanide, pronouncedly decreased the rate of exchange without altering the K content. The Q_{10} (8.5° C.–18.5° C.) for cleavage time was 2.4, and for the exchange rate of potassium 2.0. NaCN was adjusted, immediately before use, to pH 8.4 and then added to hermetically closed flasks containing egg suspension. Concentrations ranging between 1×10^{-4} to 1×10^{-3} M caused a 2–3 fold decrease in rate of exchange with complete inhibition of cleavage. The eggs showed 100 per cent recovery when returned to sea water.

[1] Department of Zoology, University of California, and Eli Lilly Research Laboratories, M. B. L., Woods Hole, Mass. Under grant from N. C. I., U. S. P. H. S.

In conclusion, before evaluation can be made of permeability differences between fertilized and unfertilized eggs, it is necessary to take into account the findings that the two types of eggs differ in their possession of relatively non-exchangeable potassium.

Cartesian diver technique: a simplified mixing method in a new type of cartesian diver vessel. C. Lloyd Claff and T. N. Tahmisian.

Problems in manipulation of Cartesian divers where solutions must be mixed after an initial trend has been established are simplified. A new type of Cartesian diver vessel is introduced which has an "hour glass" shape. It can be fashioned without difficulty in the Diver Jig described by one of us (C. L. C.) in *Science* (Vol. 107, February, 1948). The lower chamber is used as an air expansion chamber, and the alkali drop is placed therein when necessary. The upper chamber is used for one of the reaction components and the lower end of the neck is used for the second reaction component, and when necessary a third component. The oil seal is placed in the usual position. The oil is colored with Sudan III. The KOH is colored with phenolphthalein. The dyes simplify observation: Sudan III in the oil makes it possible to see admixture of flotation medium should such an accident occur.

The mixing of the reaction solutions is accomplished by over-pressure applied from a sphygmomanometer bulb and controlled by the sphygmomanometer bulb valve. The overpressure is applied to the manifold system only. The manometer proper is closed off by a three way stopcock.

Type experiments involving evolution of CO_2 from quantitative mixture of $NaHCO_3$ and H_2SO_4; normal respiration, toxicity of *Chaos chaos* to uranyl nitrate; subsequent recovery by addition of phosphate-citrate to uranyl nitrate treated *Chaos chaos;* enzymatic catalysis of CO_2 from pyruvate; fertilization of Arbacia eggs; as well as respiration of *Paramecium calkinsi,* before and after mixing of Types I and II, were studied and graphs of results presented.

Fixation and staining of plant nuclei in lacto-sudan black b. Isadore Cohen.

Sudan Black B (SBB) is soluble in 85 per cent lactic acid, giving a deep reddish-brown solution (LSBB). Chromatin is stained brown by this mixture. SBB is a hydrophobic dye and precipitates out in the presence of excess water. Fixation and staining of freehand sections of onion bulb in LSBB showed that the resting nuclei in various tissues of the plant differed in their fixation image. Nuclei with heavy coarse reticula represented one extreme and were related by a series of gradations to epidermal cell nuclei with remarkably sharp chromonematic structure. In the resting nuclei of root tips of the lima bean, the chromocenters stain red while the fine chromatin threads connected to them stain light brown.

In onion root tip smash preparations made with LSBB, late prophase, meta- and anaphase stages were as a rule so poorly preserved that they were hardly recognizable as such. The spindle was not fixed and the chromosomes appeared to be despiralized and elongated. Despiralization seems to occur also in resting and early prophase nuclei. It is suggested that this despiralization might explain the accentuated chromonematic fixation image given by 85 per cent lactic acid.

Smash preparations of onion root tips, prefixed for one-half hour in Carnoy's fluid, were made using the mixture of 2 parts of LSBB and one part of N-butyl alcohol slightly undersaturated with about 8 per cent water. Large numbers of nuclei in various stages of the mitotic cycle are thus released into the medium. Frequently, in moderately despiralized chromosomes there are seen exceptionally clear cut chromonematic structures. Nucleoli and cytoplasm do not stain. Onion epidermal cell nuclei fix and stain well in this LSBB and N-butyl alcohol mixture. When viewed with a blue daylight filter in combination with a Wratten X-1 number 11 green filter the chromosomes and nuclei appear black against a green background.

Urea reabsorption in the smooth dogfish kidney. Rudolf T. Kempton.

Urea reabsorption by the kidney tubules of the smooth dogfish, *Mustelus canis,* has been studied by the use of inulin as a measure of glomerular filtration. In most of the experiments the reabsorption of water, urea and glucose was determined simultaneously. A total of 58 collection periods in 17 different animals has dealt with urea at the normal blood levels; in 8 other collection periods with 4 animals the urea blood level was raised by the administration of very heavy doses of this substance.

There has been a surprisingly wide range of urea levels in the plasma of freshly caught animals, varying from 745 mgm. per cent to 2100 mgm. per cent urea nitrogen (including any ammonia nitrogen) or in other words urea levels from approximately 1.6 to 4.5 per cent. When the average rate of reabsorption of urea per 100 cc. of filtrate is plotted against the plasma urea level, there results a straight line which is parallel to the filtration rate. On the average, therefore, there is essentially a constant amount which is not reabsorbed from the filtrate. This does not result in a constancy of urea concentration in the urine because of the great variations in the concomitant reabsorption of water. It seems to be clear that the rate of urea reabsorption is therefore determined in some manner either by the amount of non-reabsorbed urea left in the tubule or by the plasma level. There are at present no data which give a clear indication of the proper choice of alternates, although some of the facts tend to point toward the latter as the controlling influence.

In animals in which the urea level has been raised by heavy injections of urea, there is a decrease in the reabsorption of urea in relation to the plasma level, in spite of the fact that there is no similar decrease in water and glucose reabsorption. However, in seven of the eight collection periods, if reabsorption per 100 cc. of filtrate is plotted against the plasma level prevailing before the urea injections were made, rather than afterward, the points fall in line with the other experiments. This would indicate, if further experiments now in progress substantiate this relation, that the rate of urea reabsorption has been "set" at the previous normal urea level; and that the rate did not become markedly modified during the period in which the urea level was elevated.

The influence of theophylline on the absorption of Mg-salts from the gastro-intestinal canal. A. FROELICH.

Magnesium sulphate ($MgSO_4 + 7H_2O$) is an excellent laxative. When orally administered it is not absorbed; no cases of Mg-poisoning have ever been reported.

After injection (s.c., i.m., or i.v.) a condition of "Mg-Narcosis" develops (Meltzer and Auer) easily counteracted by intravenous injection of soluble Ca-salts. When $MgSO_4 + 7H_2O$ was brought into the stomach of normal frogs (*R. pipiens*) in 25 per cent solution (ca. 0.5 cc. per 20 g. frog) nothing happened. But in frogs who had one hour before received a single injection of Theophylline-sodium-acetate (0.15 mg. pro gm.) into a lymph sac, the same amount of Mg-sulphate produced within 10–20 min. the condition of "Mg-Narcosis." Frequently the frog died within the following 24 hours. The absorption took place chiefly from the stomach and to a minor part from the rectal part of the intestine, as could be shown in frogs whose pylorus had been previously ligatured.

The Mg-chloride ($MgCl_2 + 6H_2O$) when given by mouth in a 20.5 per cent solution in a quantity similar to that of $MgSO_4$ is already absorbed from the gastro-intestinal canal, without previous injection of theophylline. This is in accordance with the findings in large mammals (sheep, goat): when big doses of $MgCl_2 + 6H_2O$ were introduced into the stomach of normal animals, this led to "Mg-narcosis."

However, experiments performed on rats during the winter of 1947–48 at the May Institute for Medical Research in Cincinnatti, O. (Director, Dr. A. Mirsky), gave results which differed in some respects from those obtained on frogs at the Marine Biological Laboratory, Woods Hole, Mass., during July and August 1948.

Both $MgSO_4 + 7H_2O$ and $MgCl_2 + 6H_2O$ were without any visible effect after introduction into the stomach of normal rats weighing from 100 to 250 gms [$MgSO_4$ in doses of 2 or 3 mg. per 50 g. rat, MgCl in isoionic quantities (10 per cent)]. After previous s.c. injection of theophylline-sodium-acetate (0.15 mg pro gm.), the sulphate when given orally produced pronounced "Mg-Narcosis" leading in some cases to the animal's death. But the introduction of $MgCl_2$ in isoionic quantities was found to be without this effect as well with or without previous treatment with theophylline. An explanation for this cannot be offered so far. For the occurrence of "Mg-Narcosis" after introduction into the stomach of both frogs and rats under the action of theophylline, increased permeability leading to absorption undoubtedly is responsible (A. Froelich and E. Zak).

The relative rate of penetration of the lower fatty acids into erythrocytes of the smooth dogfish. James W. Green.[1]

The penetration of a weak acid into erythrocytes is accompanied by a dissociation of the oxyhemoglobin to a new equilibrium. This dissociation of the oxyhemoglobin may be detected spectrophotometrically. Using a modification of the Hartridge-Roughton rapid mixing technique applicable to a spectrophotometer, a relative measure was made of the rate at which the saturated, monocarboxylic acids from formic through caprylic entered the red cells of the smooth dogfish. The method measures a chemical and not a volume change. Dilute, unbuffered red cell suspensions in sea water were mixed with dilute acid solutions also in sea water. The pH of the final mixtures was approximately 4.5 to 5.0.

The relative order of increasing rate of penetration of these acids was found to be: formic, acetic, propionic, caprylic, heptylic, caproic, butyric and valeric. This was the same order as was found for beef red cells in earlier work. The time in seconds to 50 per cent penetration of the dogfish cells was found to be: formic, 5.72; acetic, 0.89; propionic, 0.21; butyric, 0.019; valeric, 0.019; caproic, 0.054; heptylic, 0.12; caprylic, 1.44.

The times to 50 per cent penetration for acetic, propionic, butyric, valeric and caproic acids were markedly faster than the corresponding times in beef red cells. Since these acids, according to the Overton theory of lipid solubility, are thought to penetrate cells through the surface lipids it is suggested that the increase in penetration rate (over that found in beef cells for these acids) may be correlated with the greater lipid content of the fish cell ghosts as shown by the analyses of Dziemian. The penetration rate of the heptylic and caprylic acids is slower than expected on the basis of their lipid solubility. It is suggested that the molecular volume of these two acids offers a barrier to their entrance into these cells.

The effect of bacterial toxins on the permeability of dogfish erythrocytes.[2] F. R. Hunter, Jane A. Bullock and June Rawley.[3]

As one aspect of the general problem of the action of bacterial toxins on the functioning of cells, the effect of a number of toxins on the permeability of dogfish (*Mustelus canis*) erythrocytes was studied. Using a photoelectric technique to measure hemolysis times, little or no change in permeability to ethylene glycol was noted when dogfish erythrocytes were exposed to the following toxins for periods of time up to 24 hours: *Clostridium septicum, Clostridium tetani, Staphylococcus aureus* and *Corynebacterium diphtheriae*. The toxin of *Clostridium perfringens* caused a marked decrease in hemolysis time in ethylene glycol almost immediately (less than 10 min.). The toxins of *Bacillus cereus* and *Streptococcus pyogenes* caused a decrease in hemolysis time after several hours (6–10 hours) exposure. It is hoped that a further analysis of these data and a comparison with comparable data obtained from studies on chicken erythrocytes, *Asterias* and *Arbacia* eggs may give some information as to the action of toxins at the cellular level.

New experiments and observations on sexual instability in Crepidula plana. Harley N. Gould and Sidney C. Hsiao.

This report involves further attempts to clear up the mechanism of self-regulation of sex in communities of *Crepidula plana*. In isolation-experiments, some of the small, sexless individuals confined in separate glass tubes in the harbor water at Woods Hole have developed a temporarily functional male phase, as found by Coe, while control cultures in running sea water in the laboratory have disclosed at most only a partial male development. The difference does not appear to be of nutritional origin, as suggested by Coe, because (a) cultures in running sea water show equal or greater rapidity of growth compared with identical cultures in the harbor, and (b) young sexless individuals confined in tubes with females in running sea water in the laboratory develop rapidly into adult males.

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[1] Physiological Laboratory.

[2] This work was supported by grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service and the Faculty Research Fund, The University of Oklahoma.

[3] Marine Biological Laboratory, Woods Hole, Mass., and the Department of Zoological Sciences, The University of Oklahoma, Norman.

Congenital differences in growth rate and sexual development appear in every culture of equal-sized young. This forms the basis for the presence of both sexes in the community, which is later assured by the effect of the more rapidly growing individuals on the smaller ones.

Individuals of comparatively rapid growth under isolation, apparently tending toward direct female development, can be induced to develop into adult males by associating them with immature or mature females.

Males which after long isolation have lost external male characters have regained full male development after eleven days association with females, and sections of the visceral sac demonstrate evidence of internal sexual atrophy followed by regeneration.

In one-fourth of the cases, males from which the phallus has been removed by operation, when kept in association with females, regenerate the organ completely, and in another one-fourth, partially, after nine days. Males isolated after the operation show much less regenerative ability.

These experiments are continuing, and investigations of starvation and of hormone effects are under way.

The doubtful character of "break" excitation in skeletal muscle. Paul G. LeFevre.

It was previously reported that nerve-free regions of the frog sartorius and pharyngeal retractor of *Thyone* failed to respond at "break" of a constant current. This was in accordance with absence of accommodation in these tissues, in light of excitation theory. Since this report, several papers from Dittler's laboratory at Marburg have dealt with response of curarized muscles to interruption of a steady current.

For this reason, the matter was briefly reinvestigated on gastrocnemii from frogs, in some of which a section of the sciatic nerve in the lower thigh had been removed 2–4 weeks previously. These muscles were stimulated through non-polarizable calomel half-cells by means of a specialized generator of pulses of about 2 seconds' duration; the automatic keying, tested oscillographically, was free from disturbing transients. The isometric response was observed oscillographically by amplification of current from a Statham strain gauge attached to the tendon of the slightly stretched muscle.

Muscles containing intramuscular nervous elements responded in the classical manner, with a sharp development of tension at "make" and "break" of the current and some degree of maintained tension during its passage, depending on current intensity. "Break" response typically occurred with current intensities over 3–5 times threshold for "make" response. In denervated muscles, no "break" response was seen even at intensities up to 15 times "make" threshold. The "break" response was not, however, simply attributable to involvement of intramuscular nervous elements, as it persisted in muscles exposed to *d*-tubocurarine in doses blocking neuromuscular transmission.

Since the capacity for "break" response appears to be neither intrinsic in denervated muscle nor dependent on neuromuscular transmission, it is suggested that it is a property of a specialized region of the muscle at the neuromyal junction or of a special structure there interposed.

Comparison of frog nerve and squid axon with respect to the measurement of accommodation. Paul G. LeFevre.

The previous report of the occurrence of "break" response in the absence of accommodation, in decalcified frog nerves, was based on observation of the muscular response as index of nerve activity. Some difficulties of interpretation were avoided by use of a single-unit system, the squid giant axon, in an effort to check the phenomenon; but it proved impossible to alter the natural rate of accommodation in this cell by decalcification. The unaltered accommodation rate in the face of pronounced autorhythmic activity in the decalcified state invalidates the simple interpretations of this rhythmicity suggested by Solandt and Katz.

The pattern of response of the giant axon to alternating currents failed to conform with excitation theory. Curves relating liminal intensity with the logarithm of the frequency were symmetrical about the optimal frequency, in accordance with Coppée's observations on other tissues; but there was consistent deviation from the predicted linearity in the relation between the squares of the liminal intensity and of the periods above the optimum.

The accommodation rate was extremely rapid in the giant axon; measured by stimulation with alternating currents, Hill's λ was not definitely measurable due to the deviation from theory, but was certainly less than 7 msec.; measured by stimulation with exponentially rising currents, $\lambda = 0.9 - 1.5$ msec., or only 2–4 times the factor for the excitatory process; whereas in frog nerve the two factors are of entirely different orders of magnitude.

The disagreement between the rates of accommodation measured on the giant axon in these two ways (which yield identical results in frog nerve), and the non-conformity of the squid axon with theories fitting the behaviour of the frog nerve are probably associated with observations on giant axons by Arvanitaki, Cole, and others, indicating that the response of the local excitatory state to subthreshold electrical disturbances is considerably more complex than as postulated in the excitation theories of Rashevsky, Hill, and Monnier.

August 25

Do genes exist? P. W. Whiting.

Johannsen named the gene and supposed it to be a "unit factor" or "element" "demonstrated by modern mendelian researches" and he suggested that it might be a side-chain of a large protein molecule. Recent speculation follows the same course, the gene being thus a corpuscular unit tracing back to the micella, the pangene, etc. It is here suggested that the word genes be used for terms in genetic formulae expressing mendelizing differences. It follows from this, somewhat in agreement with Goldschmidt's dissenting views, that the physical basis for genes is a considerable variety of chromosomal conditions. The germ plasma is genic material but it does not consist of genes. It produces genes by such structural reorganizations as may subsequently mendelize with the original condition. Usually this reorganization is very localized, giving rise to the linear order of the genes, but it may involve many regions provided these act as a unit of segregation in meiosis of the heterozygote. There is, however, no basis for assuming unified segregation in the homozygote. For example, the sex-differentiating gene pair of Drosophila mendelizes in the male, the heterozygote Mm (YX), but there is crossing-over between homologous parts of paternal m and maternal m in the female, mm (XX). The physical basis for the dominant gene M is the absence of one of the X chromosomes. Similar reasoning may be applied to gene pairs whose physical basis is known to be located in inversions, deletions, repeats, etc. Furthermore it seems preferable to extrapolate the nature of mendelizing pairs or series which are not microscopically demonstrable from those that are rather than to postulate the existence of a genic corpuscle by analogy with the subcellular units of past philosophies.

Dominant lethals induced by x-rays in sperm of the chalcidoid wasp Nasonia brevicornis Ashmead [= *Mormoniella vitripennis (Walker) fide Muesebeck in lit.*]. D. T. Ray.

In preliminary tests, 1287 ♀♀ and 249 ♂♂ (84.4% ♀♀) were bred from 8 mated mothers, 2097 ♂♂ from 11 unmated. Later 2117 ♂♂ were bred in 41 egg-laying days (51.6 per day) from 13 young unmated mothers and 1042 ♂♂ in 32 days (32.6 per day) from 4 ♀♀ exposed to males. In the x-ray experiment males were treated as freshly eclosed adults and were then paired with untreated females. The females were set with several blow-fly pupae and transferred every two or three days for three or four transfers. 119 egg-laying days of the controls yielded ♀♀ 4213 (35.4 per day), ♂♂ 911 (7.7 per day) (82.2% ♀♀). Data for the treated arranged as r units/egg-laying days/daughters/sons were 500 r/40/1013/270; 1000 r/49/1190/319; 1500 r/45/673/314; 2000 r/128/1326/903; 2500 r/16/95/133; 3000 r/49/341/404; 3500 r/24/84/245; 4000 r/90/200/700; 5000 r/60/50/540; 6000 r/128/24/1142; 7000 r/53/4/365; 8000 r/52/1/386; 9000 r/42/0/434; 10,000 r/34/0/342. Curve for ♀♀/total progeny is much steeper than that for Habrobracon (Heidenthal, *Genetics*, **30**: 197–205) but not as steep as that for Melittobia (Kerschner, *Anat. Rec.*, **96**: 556) because sex ratio of the Nasonia controls is intermediate. A close fit to the Habrobracon curve is obtained by multiplying by 70/82, the percentage of Habrobracon females in controls divided by that of Nasonia. Sons, averaging 7.97 per day, are not directly affected by increasing dose, but with higher treatments of their "stepfathers" they show a slight increase in numbers and a considerable increase in size due to fewer sisters competing for food. Excluded from the above data are males produced after presumed exhaustion of the mothers' sperm supply. These totalled 2681 bred in 72 days, the average 37.2 contrasting with the low numbers appearing in earlier vials. Also excluded are 5 vials of diapause larvae produced by 2 mothers preceding and by 3 mothers following production of progeny with normal life span.

The use of diethylstilbesterol in the production of eye mutations in drosophila melanogaster. BURTON L. STEKLER.

The use of nitrogen mustard in the production of genetic mutations in *Drosophila melanogaster* is well known. However, there is no specificity in its action and like X-ray, produces the random type of mutation. In work along similar lines of investigation, it was found that diethylstilbesterol produced striking changes in the architecture of the Drosophila eye, and that these changes could be reproduced in succeeding generations.

Due to the insolubility of diethylstilbesterol in water, the ordinary corn meal-molasses medium was not used. Instead, a synthetic medium containing sucrose, inorganic salts and agar was substituted. The drug, in varying concentrations (1 mg.%–20 mg.%), was then added, and the food allowed to harden. Three or four drops of a water-yeast mixture were added to this.

Three different wild type stocks of Drosophila were used for the experiment. Two males and two females of each stock were allowed to mate on the drug-containing medium. Eggs were laid thereon and the larvae were forced to eat the drug-containing medium. The temperature was kept at 24° C. after the parents were put in with the experimental medium.

The F–1 generation was studied for mutations and it was found that the drug-treated Woodbury and Florida wild type flies exhibited a fairly regular eye mutation. The eye characters resembled lobed eye, but as yet this fact has not been proven. The percentage of mutants in the two wild type stocks varied between 0.4–0.7%. The Oregon wild type stock did not produce any mutations at 24° C. but did so at 29° C. Control groups of flies were run under the same conditions and the mutants were not observed to appear in 3500 individuals. These results substantiate previous data which were collected in 1946, in which the same experiment was tried. Mutants produced at that time have been carried on normal food since the F–1 generation and have so existed for 56 generations.

There are indications pointing to the Stilbene nucleus as being the important factor in this chemically produced mutation. This is shown as a result of adding stilbamadine, which is closely allied in chemical structure to diethylstilbesterol, to the synthetic medium, the same eye changes are produced, only at a greatly reduced frequency.

Much remains to be done in proving whether or not this drug is specifically directed and investigations will be continued toward this end.

Predictable mutations in bacteria. E. RUTH WITKUS.[1]

A colorless mutation has been produced in *Sarcina lutea*. The colorless mutation was obtained by mixing several different organisms in a liquid medium and growing the organisms together for twenty-four hours at 37° C. The following four species of bacteria were grown together in nutrient broth for twenty-four hours: *Bacillus subtilis, Bacillus megatherium, Proteus vulgaris* and *Sarcina lutea.*

After twenty-four hours the organisms were reisolated by the dilution method and five different strains instead of four were recovered. In addition to the four original species, a new non-pigmented form was always obtained. The new type is similar to *Sarcina lutea* in all morphological features except in its color. The yellow and white colonies appeared in a ratio of three to one.

If *Proteus vulgaris* was omitted from the mixture, a white mutant was also produced. If either *Bacillus megatherium* or *Bacillus subtilis* was omitted, no white mutant was produced. No white mutant was obtained when mixtures of only two organisms were used.

A mixture of four entirely different organisms was used—*Bacillus cereus, Corynebacterium xerose, Serratia marcescens* and *Sarcina lutea.* No white mutant was obtained in this instance.

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[1] Biological Laboratory, Fordham University.

White mutants were also obtained by growing *Sarcina lutea* in a liquid medium containing a sodium salt of ribose nucleic acid.

Method of origin of androgenetic males in Habrobracon. ANNA R. WHITING.

1.57 per cent of eggs x-rayed in first meiotic metaphase and fertilized by untreated sperm develop into normal fertile haploid androgenetic males. Although lethal dose for the egg nucleus in this stage is about 2,400 r, androgenetic males occur after treatments as high as 54,000 r. Of 291 eggs in which origin of cleavage nuclei could be determined, 3 or 1.03 per cent showed cleavage of sperm nuclei only. In 3 others, incipient androgenesis was strongly suggested. The maximum number of 6 or 2.04 per cent compared with 1.57 per cent of adults emerging shows that androgenetic larvae do not differ in viability from larvae developing in untreated eggs. Chromatin bridges in meiotic division II which occur following treatment in metaphase I allow androgenetic development when they retard the egg nucleus to such a degree that it does not reach the sperm nucleus before cleavage begins. The rarity of such bridges in eggs following treatment in prophase I will explain why no androgenetic males developing from these eggs have been found.

X-Radiation effects on the restitution of dissociated Microciona. C. K. LIU.[1]

Dosages of 10,000 r, 25,000 r, 36,000 r and to some extent 50,000 r, given to *Microciona* immediately before squeezing it through bolting silk with meshes of 40 μ, produced little effect on the process of restitution.

Dosages of 100,000 r to 300,000 r were excessive. With 300,000 r, aggregation still occurred but stopped at the spheroid stage and the aggregates died after 4 days. With 100,000 r and 200,000 r aggregation proceeded to an ill-defined reticulum stage. The cell mass then retracted from the surrounding hyaline layer and collected into groups of rounded-up cells. Some of the masses survived for 3 weeks, but no differentiation took place.

The dosage of 72,000 r produced significant results and was studied in more detail. Irradiation was done on: (1) sponges just before being dissociated; (2) suspension immediately after dissociation; (3) restituted sponges 10 days after dissociation when large numbers of flagellated chambers were formed.

In (1) and (2) aggregation occurred normally but, after the reticulum stage, retracted instead of going into the stage of spreading. The retracted condition persisted until the 11th day when the spreading out began to occur. Flagellated chambers appeared on the 14th day instead of on the 4th day as in the control.

As an immediate result of (3) many flagellated chambers actually disappeared, presumably due to their breaking down into separate choanocytes. The remaining chambers collapsed. There was a decided loss of definition of all cells. Retraction of the colony soon followed and showed 50 per cent reduction in size at the end of 24 hours, leaving spicules behind. The colony retrogressed into a mass of cells with no structural differentiation. The nucleolated archeocytes became filled with coarse orange granules, presumably ingested choanocytes. The colony remained retracted for 10 days when it again spread out. Flagellated chambers began to form on the 14th day. Their subsequent development showed no superiority in number or size over (1) and (2).

By comparing (1), (2) and (3), one can conclude that the regeneration of the flagellated chambers in the irradiated material is independent of their state of development at the time of irradiation. The fact that the flagellated chambers formed at the same interval of time after irradiation and increased at the same tempo suggests that the choanocytes are formed *de novo* from the archeocytes.

Combined effect of ultraviolet light and heat upon first cleavage of Arbacia eggs. LOIS M. HUTCHINGS.

Ten minutes after insemination, Arbacia eggs were exposed for 1, 2, 3, 4, or 5 minutes to a temperature of 36° ± 0.05° C. or were given 40, 80, 120, 160, or 200 seconds of 2537 Å ultraviolet light. Other eggs were given serial dosages of heat preceded or followed by 10 seconds exposure to ultraviolet light, and still others received serial dosages of ultraviolet light preceded or followed by 120 seconds heat at 36° C. Thus, in addition to controls there were six phases to a complete experiment. All dishes were kept on a water table whose temperature variation during the experiment was ± 0.15° C. The time at which 50 per cent underwent first cleavage was determined through several counts of 100 eggs per count.

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[1] Laboratory of Experimental Cell Research, M. B. L., Woods Hole.

Thermal treatment applied to Arbacia eggs in serial dosages shows a continuous progression from mild to severe inhibition, which increases markedly after 3½ minutes of heating. Serial dosages of 2537 Å ultraviolet light produce an intense initial inhibition of cleavage followed by a mild secondary inhibition which increases slowly in proportion to dosage. Treatments involving both irradiation and heat inhibit cleavage more than either agent alone. Curves of treatments involving irradiation and serial dosages of heat show no significant difference from a hypothetical curve representing the sum of the effects of each agent alone. Curves of treatments involving heat and serial dosages of ultraviolet light almost coincide with a hypothetical curve representing ⅞ of the sum of the effects of each alone. There is no evidence that irradiation preceding heat causes an exaggerated injury which application of the same agents in reverse order fails to do, i.e. there is no sensitization to heat through ultraviolet irradiation.

Streptomycin-induced chlorophyll-less races of Euglena. Luigi Prova~~losi~~soli, S. H. Hu~~nter~~tner,[1] and Albert Schatz.[2]

Several aseptic clones of *Euglena gracilis, E. gracilis* var. *bacillaris* and var. *urophora* became permanently apochlorotic in light through the action of streptomycin. Detailed investigations were made of bleaching as a function of concentration and duration of exposure to streptomycin, sensitivity of proliferating and non-proliferating cells and comparative sensitivity in light and darkness.

The following results were obtained.

There was a smoothly progressive bleaching of the cultures with increasing duration of exposure and increasing concentration of streptomycin.

There was no difference either in the intensity of bleaching between proliferating and non-proliferating cultures or in the time of exposure to streptomycin required, nor between light- and dark-treated cultures under proliferating conditions, in these respects.

There was no evidence of development of streptomycin dependency.

With streptomycin constant at 100 μg/ml., an exposure of 1–8 hours was required for loss of roughly 50 per cent of the color, and 4 or more days for apparently complete bleaching.

With constant time of exposure (15 days), 1 μg/ml. gave roughly 50 per cent loss of color, and bleaching was nearly complete with 40 μg/ml.

Streptomycin did not interfere with the utilization of carbon sources in darkness.

Preliminary microscopic observation of partially bleached mass cultures showed them to consist of both pale and almost fully green individuals. However a few green cells had less thick chloroplasts or only 1–2 ill-defined chloroplasts instead of the usual number. Aged white cells, derived both from primary treated cultures and from subcultures, accumulated an unusually large number of red granules.

The stigma and paramylon grains remained permanently unaltered in all colorless cultures.

The bleached clones have been carried through ten transfers in light without showing any resumption of color.

The development of the basal mat in Hydractinia. Sears Crowell.

Small fragments of the basal mat of Hydractinia to which a few hydranths are attached can be isolated and fed with the nauplii of the brine shrimp, Artemia. These fragments sometimes grow by the extension of stolons. However, in many cases, instead of producing stolons, the edge of the original mat grows out as a whole. Previous descriptions of the mat of Hydractinia state or imply that it arises and grows by the production of stolons which later anastomose and broaden to give a solid mat. It is clear in my specimens that the mat may grow directly at its edge without an intermediate stage of stolon proliferation and subsequent fusion.

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[1] Haskins Laboratories.

[2] Sloan-Kettering Institute for Cancer Research.

Specificity in the fusion of stolons in hydroids. SEARS CROWELL.

The growth and fusion of stolons arising from small fragments of colonies of Hydractinia, Podocoryne, and Stylactis have been observed. When stolons growing out from the same fragment or from separate fragments taken from the same colony meet, they fuse in nearly every instance. However, fusion following contact does not occur between stolons of different genera, of different sex within a species, nor of different colonies of the same species and sex. It is clear that there is individual specificity in the fusion of stolons in these three hydroids.

The distribution of the cerebrospinal fluid in the lower vertebrates. H. P. K. AGERSBORG.[1]

Although a great deal of research has been done in anatomical laboratories in various countries on the distribution and function of the cerebrospinal fluid in some of the higher vertebrates (dog, cat, rabbit, man), the results of this type of research are not generally known among biologists. Since it is to the biologist the physician usually turns for information on basic biological problems, it was thought quite pertinent to tackle a study of this nature in a biological laboratory. It was also thought that if the cerebrospinal fluid is of such great importance in man as the members of the healing arts think, it must be of like importance in not only mammals generally, to which the medical practitioner has turned for experimental enlightenment, but also to the vertebrates as a group of which the mammals including man are a part. For these reasons we have started a study of this problem in the lower vertebrates, using the same methods as have been used by the experimental worker in medical schools on higher vertebrates (mammals), and thinking that if the cerebrospinal fluid is of such great importance to mammals as the members of the healing arts claim, it must be of importance to vertebrates other than mammals, even to the lower vertebrates. If so, we would expect that such a system would be common to all just as the other visceral organs are not only analogous but homologous in all the vertebrates. Homologous organs are such that they are the same in origin and structure. Such is by and large the central nervous system in vertebrates. Therefore, this should hold, regarding the relationship of the nerves and their investments to the central nervous system on the one hand, and to the peripheral terminals on the other. This is exactly what we have established, using elasmobranch and teleost fishes, the frog, and turtle. There is a universal phylogenetic principle prevailing, a principle of common morphology, and perhaps also a common function. To our knowledge, no one has studied this problem in the lower vertebrates before.

Implications of cerebrospinal fluid distribution in the therapy of the healing arts. PAUL E. KIMBERLEY, D.O.[1]

The relationship between the cerebrospinal fluid and the rhythmical pulsations of the brain has been developed to formulate a functional unit. This unit, among other possibilities, aids the movement of cerebrospinal fluid from the intercellular spaces into the perivascular and subarachnoid spaces, thus simulating the mechanisms for moving intercellular fluids in other parts of the body.

The distribution of cerebrospinal fluid, as it has been demonstrated, to the olfactory mucosa, the eye, the inner ear, the visceral nerves including their ganglia and the craniospinal nerves is enhanced and probably motivated by the pulsations of the central nervous system tissue. Thus a factor for promoting the biological requisites of fluid motion has been added which previously was not discussed for the nervous tissues.

The possibility of stasis of cerebrospinal fluid naturally arises when considering the causes for the physiological perversions known as symptoms of disease.

The effect of such a mechanism is seen as a possible factor in the work of investigators using the vertebrates, especially mammals.

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[1] Des Moines Still College of Osteopathy and Surgery, Des Moines 9, Iowa.

PAPERS READ BY TITLE

Studies of the chemical form of P^{32} after entry into the arbacia egg. DR. P. H. ABELSON.[1]

Further studies [2] have been made of the P^{32} uptake by eggs of the *Arbacia punctulata,* 1–5 hours after fertilization. When eggs are exposed to sea water containing 10^{-8} to 10^{-9} grams of inorganic phosphate and .02 μc. of P^{32} per ml., an uptake of P^{32} is observed.

The new work shows that at least 97 per cent of the P^{32} appears in the egg as tri-chlor-acetic acid soluble compounds. An attempt was made to determine the chemical nature of these compounds. Procedures outlined by Umbreit [3] et al. were employed yielding barium soluble and barium insoluble fractions. The latter fraction was further divided into an inorganic phosphate component and a precipitate containing ATP and ADP. From hydrolysis studies of the barium soluble material, it is clear that this fraction contains at least two components. Thus one finds at least four chemical forms of P^{32} very soon after entry of the substance into the egg. The total P^{32} increases linearly with time from 1–5 hours after fertilization and the various acid soluble fractions likewise seem to increase linearly.

The difficulties of performing P^{32} chemical analysis in the presence of echinochrome proved enormous. By using small amounts of eggs (.1–.2 cc) and large amounts of carrier, 4 mg. P and 30 mg. ATP, it was possible to obtain more satisfactory separations. The average of twelve determinations performed without ATP carrier gave the following percentages of acid soluble components:

PO_4	ATP & ADP	Ba soluble	Missing
42	23	21	14

With carriers for inorganic phosphate and ATP the results of six determinations gave:

PO_4	ATP & ADP	Ba soluble	Missing
16	41	41	2

It is possible that these results might be altered if carrier for the barium soluble components were available.

Penetration of radioactive phosphate into the eggs of Strongylocentrotus purpuratus, S. franciscanus, and Urechis caupo. S. C. BROOKS AND E. L. CHAMBERS.[4]

Samples of a 0.2 per cent suspension of (1) unfertilized and (2) fertilized eggs at 15° C., containing P^{32} as orthophosphate at concentrations of 0.001–.30 μc/ml., were centrifuged in Hopkins vaccine tubes. The supernatant was withdrawn and the volume measured to an accuracy of $\sigma = .00015$ ml. The radioactivity of each sample was determined. Correction was made for the activity of the remaining supernatant.

In the unfertilized egg the rate of penetration is slow and remains approximately constant for hours.

In the fertilized egg a definite increase in penetration occurred within the first 6 min. and the rate rapidly rose after 15 min., reaching a maximum by 60 min. (cleavage at 110 min.). After 60 min. the rate remained constant throughout the first three cleavages.

At total initial orthophosphate concentrations of 1.4×10^{-5}, 4.3×10^{-6}, and 2.3×10^{-6} GM/liter the "penetration" constants for the unfertilized eggs were respectively 2.2×10^{-12}, 1.85×10^{-12} and 2.0×10^{-12} GM per ml. of eggs per second while for the fertilized eggs the values were 2.9×10^{-10}, 2.0×10^{-10} and 1.65×10^{-10} GM/ml./sec. In *S. franciscanus* the values at an initial suspension concentration of 1.7×10^{-6} GM/liter were: for the unfertilized eggs, 7.4×10^{-13} GM/ml./sec.;

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[1] Carnegie Institute.

[2] Abelson, P. H., *Biological Bulletin,* **93**: 203 (1947).

[3] *Manometric techniques,* Burgess Publishing Co.

[4] Department of Zoology, University of California. Under grant from the N. C. I., U. S. P. N. S.

and for the fertilized, 8.2×10^{-11} GM/ml./sec. Expressing results in terms of GM/ml. of eggs/sec./GM per liter of suspension, the rate of entry into the fertilized eggs of both species was found to be 130–160 times that of the unfertilized.

Continuous washing of fertilized eggs failed to remove more than 2–5 per cent of the contained activity. Therefore, the penetration constants for the fertilized egg essentially represent the rate of increase of total P in the egg, namely, only about 0.1 per cent increase in total P per hour. NaCN at 1×10^{-4} M decreased the rate of penetration threefold.

Eggs of *Urechis caupo* show a similar phenomenon but the increase in rate does not occur until after the second cleavage.

The evidence indicates that (1) the rapid penetration of orthophosphate in the fertilized egg represents an accumulation rather than an exchange; (2) the fraction of orthophosphate entering the fertilized eggs is greater the lower the suspension concentration; (3) during the first three cleavages the rate remains constant irrespective of the mitotic and cleavage cycle, and increase in surface area.

A preliminary account of this work was given at the 28th Annual Meeting of the Pacific Division of the A. A. A. S., San Diego, June 18, 1947.

Distribution of radioactive phosphate in the eggs of the sea urchin Lytechinus pictus. E. L. CHAMBERS, A. WHITELEY, R. CHAMBERS AND S. C. BROOKS.[1]

A single layer of eggs was continuously perfused with sea water containing P^{32} at 0.0005 μc/ml. The eggs were microscopically observed from above, and counting rates taken with a Geiger-Muller tube placed under the chamber. Eggs fertilized in the chamber showed the same uptake curve as described above for *S. purpuratus.*

Samples of membrane-free fertilized eggs in P^{32} sea water were washed in a NaCl/KCl mixture to remove the hyaline plasma layer, and the activity measured. The rate of penetration into these denuded eggs was the same as in the controls.

Eggs containing P^{32} were centrifuged into light and heavy fragments. The equal sized portions of unfertilized eggs showed equal activity per ml. Membrane-less fertilized eggs separate into large, coarse granules and small heavy hyaline portions containing fine granules which stain with methyl green. The activity per ml. of the latter was twice that of the former.

Eggs containing P^{32} were washed and homogenized at different stages of development at 0° C. in 5 per cent trichloracetic acid and the activity of the acid soluble (AS) and acid insoluble (AI) fractions determined. In all cases (unfertilized and fertilized up to 16 celled stage) the activity in the AI fraction varied from 3.5–4.1 per cent of the total. Fertilized eggs exposed to P^{32} from 0′ to 60′, then washed free and homogenized at the blastula stage, showed 6.9 per cent activity in the AI fraction. Activity in the phospholipid fraction of the AI was negligible. Killed eggs homogenized in the presence of P^{32} showed 0.85 per cent of the total activity of the suspension in the AI. This is far below the percentage of activity accumulated in the AI fraction of living eggs.

P^{32} accumulates progressively after fertilization and tends to be more intensely segregated in certain portions. After homogenization in trichloracetic acid the activity is found primarily in the AS fraction. A significant percentage of the activity is found in the AI fraction, which increases during development.

On the combining weight of Cypridina luciferin. AURIN M. CHASE.

Giese and Chase (*J. Cell. Comp. Physiol.*, **16**: 237, 1940) calculated a combining weight for partially purified Cypridina luciferin on the basis of its reaction with cyanide. Their value was between 800 and 2400, the lower value seeming more probable. Because of certain sources of error inherent in their method, a redetermination by some other method seemed desirable. The method chosen depends upon the oxidation of luciferin by ferricyanide (Harvey, *J. Biol. Chem.*, **78**: 369, 1928; Anderson, *J. Cell. Comp. Physiol.*, **8**: 261, 1936).

Having established that potassium ferricyanide in low concentrations was without significant effect upon the enzyme, luciferase, the luminescent reaction was measured over a range of ferricyanide concentrations from 10^{-5} to 10^{-9} Molar. The luciferin was from two cycles of purification by Anderson's method (*J. Gen. Physiol.*, **19**: 301, 1935). In one series of experiments the oxidant acted upon the luciferin for one-half minute; in another series, for five minutes. In all cases 0.0016 mg. of the product from the purification procedure was present in 10 ml. of pH 6.70 phosphate buffer containing the desired concentration of ferricyanide.

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[1] University of California, California Institute of Technology, and M. B. L., Woods Hole, Mass.

The percentage of reduced luciferin decreased progressively from 100 per cent, at a ferricyanide concentration of 10^{-8} *M*, to zero per cent at a concentration of about 3×10^{-7} *M*. Using this latter figure and assuming that the luciferin was free of impurity (the degree of purity is not actually known but is probably fairly high) a value of about 500 was calculated for the combining weight. If the oxidation as measured involves a one electron change, which may be the case in the particular procedure used, the molecular weight would be equal to the calculated combining weight, about 500. For a two electron change it would be double this value.

Fat cell size in the mutant small-wings of Habrobracon. A. M. Clark and D. S. Grosch.

The presence of the gene small-wings (sw) reduces the length of wings and the size of wing tissue cells. In order to determine whether this gene also affects other types of cells in the body, measurements of "fat cells" were undertaken. Conditions of measurement as outlined by Grosch (1948—*Proc. N.C. Acad. of Science*) were that the animals be equivalent in size, that they be used immediately upon emergence, and that isosmotic Ringer's solution be used as the suspending fluid. Five animals from each of the six genotypes obtained from $\frac{c\ +}{c\ sw}$ ♀ × + sw ♂ were selected for the present study. The abdomen of each was removed and dissected on a slide containing a drop of Ringer's in order to permit dispersion of the fat cells. Diameters of one hundred fat cells were measured from each animal.

Genotype	$\frac{c\ +}{+\ sw}$ ♂	$\frac{c\ sw}{+\ sw}$ ♂	c + ♂	c sw ♂	$\frac{c\ +}{+\ sw}$ ♀	$\frac{c\ sw}{+\ sw}$ ♀
Mean Fat Cell Diameter ± S.E. in microns	107.36 0.66	103.51 0.66	102.52 0.55	97.57 0.66	98.01 0.66	87.34 0.55

Comparison of diploid males $\left(\frac{c\ +}{+\ sw}\right.$ ♂$\left.\right)$ with diploid males $\left(\frac{c\ sw}{+\ sw}\right.$ ♂$\left.\right)$, haploids (c + ♂) with haploids (c sw ♂), and females $\left(\frac{c\ +}{+\ sw}\right.$ ♀$\left.\right)$ with females $\left(\frac{c\ sw}{+\ sw}\right.$ ♀$\left.\right)$ indicates that the mutant small-wings effects smaller fat cells. These size differences are similar in trend to those obtained for the wing cells (Clark and associates—unpublished). Further, comparison of diploid males with haploid males indicates that the diploid males have larger fat cells. This also can be correlated with wing cell size. Comparison of males and females shows a sex difference in that females tend to have smaller fat cells. This sex difference is consistent with findings by Grosch on stock No. 25 and on stock No. 33 which are wild-type except for eye color markers.

The relation of the plasma membrane, vitelline membrane and jelly in the egg of Nereis limbata. Donald P. Costello.

The relation of the plasma membrane to the extraneous coats and cortical layers of the Nereis egg may be ascertained by observation of the normal living egg and of the egg after certain experimental treatments. Evidence obtained from experiments with the centrifuge and by treating the egg with alkaline sodium chloride indicates that the plasma membrane of the unfertilized egg is external to the jelly precursor granules of the cortex, and just inside the vitelline membrane. Experiments with alkaline sodium chloride (pH 10.5) indicate that the perivitelline space of the fertilized egg is extra-ovular after jelly extrusion is complete. The behavior of the egg in alkaline sodium chloride and the normal cortical response of the egg to fertilization are attributed largely to the properties of the jelly. Preliminary data (determinations by Ferry, 1939) indicate that it contains less than 1 per cent nitrogen, and is at least 75 per cent carbohydrate (estimated by the Tillmans-Phillipi method, and corrected for ash). The jelly is precipitated in fibrous form by barium ions at alkaline reactions but not at neutrality. The evidence suggests that the jelly is a uronic acid polymer, occurring as the calcium (and perhaps magnesium) salt.

Spiral cleavage is generally assumed to be limited to the polyclad Turbellaria, the Nemertea, the Annelida and all Mollusca except the Cephalopoda. Actually, spiral cleavage is considerably more widespread, if one considers certain modifications of the typical oblique cleavage taking place in four quadrants. In all forms, spiral cleavage becomes modified into bilateral cleavage at some stage in development.

As described by Bresslau (1909, 1928) and Costello (1937), the Acoela, during early cleavage, are characterized by oblique spindles which alternate in their laeotropic and dexiotropic positions to give off three duets of micromeres. The developing egg thus consists of only two hemispheres, in contrast to the four quadrants typical of the Polycladia, Annelida, and Mollusca.

A further deviation is exemplified by Lepas and other Cirripedia. Cleavage of Lepas may be looked upon as a cleavage by "monets," or as unit cleavage. If this unit is designated to correspond to the D-quadrant, three micromeres (1d, 2d, 3d) are given off in succession to give rise to ectoblast and secondary mesoblast, and the fourth micromere given off (4d) is the primary mesoblast. The earliest cleavages have spindles which are definitely oblique. Bigelow (1902) has used the notation of a quadrant system to designate the cleavage products, but he realized that he was not dealing with a four-quadrant system. This was in disagreement with the comments of Mark and Castle, who considered Lepas to have a slightly modified four-quadrant cleavage.

Induction of autogamy in single animals of Paramecium calkinsi following mixture of two mating types. WILLIAM F. DILLER.

Mixtures of two mating types of *P. calkinsi*, obtained through the courtesy of C. B. Metz, give rise to autogamous reorganization in single animals, in addition to normal conjugating pairs and "pseudo-selfing" clumps of a number of reorganizing individuals, loosely joined together. The latter are presumably similar to those resulting from mixture of living animals and dead animals of the opposite mating type of *P. aurelia* (Metz, '47). This race of *P. calkinsi* is characterized by a single micronucleus. A fungoid symbiont of the macronucleus is frequently found. Nuclear activity in autogamy in single animals of *P. calkinsi* closely resembles the nuclear phenomena of conjugation and both are much like the corresponding processes in *P. aurelia*. Typical crescent stages are rarely, if ever, found in single autogamous individuals. This is very different from the conditions in *P. aurelia* (Diller, '36) and *P. polycaryum* (Diller, unpublished data). Large swollen first meiotic prophase nuclei are often seen. Each of the two young macronuclear anlagen in both ex-conjugants and autogamous animals contains about thirty deeply staining granules which are probably enlarged, modified chromosomes. This point is being investigated further.

An extra post-zygotic division in Paramecium caudatum. WILLIAM F. DILLER.

Three post-zygotic divisions following fertilization have been considered the normal procedure in the conjugation of *P. caudatum*. In a race from a Philadelphia pond in which the conjugants separate normally in a late stage of the division of the synkaryon, evidence has been secured indicating that four post-zygotic divisions occur, though only one of the nuclear products of the first division takes part in all of them. One of these sister nuclei degenerates, while the other divides three additional times to produce eight nuclei, four of which become macronuclear anlagen. This extra division, with degeneration after the first, agrees with Chen's recent findings in *P. bursaria*.

Further observations on the metabolism of clams' tissues in sea water at different salinities. HOYT S. HOPKINS.

Earlier results (*Anat. Record*, **96**: 522, 1946) indicated that the stimulating effect of diluted sea water upon oxygen consumption of excised gill tissue of *Venus mercenaria* was not primarily due to reduced concentration of any specific cation of sea water. The higher rate of respiration in ⅗ sea water was maintained when the concentration of K or Ca was restored to that in natural sea water, without raising the osmotic pressure. Replacement of Mg salts caused a reduction of one-third in the respiratory increment above that in normal sea water.

Recent experiments with Van't Hoff's solution led to a similar conclusion: that the dilution effect is not the result of a reduction in concentration of particular salts. In this artificial medium the oxygen consumption of gill was slightly lower (9 per cent) than in natural sea water. The rate increased over 40 per cent in Van't Hoff's solution diluted to ⅗, which was about as much as in diluted natural sea water. When Mg salts were omitted from the solution, maintaining the same osmotic pressure, there was an increase in oxygen consumption of about 20 per cent. If half of the Mg was added—giving a concentration approximating that in the diluted sea water experiments—the rise in oxygen consumption was negligible. Since the respiration of gill was augmented in diluted sea water, but not when isotonic NaCl, sucrose, or glucose solution replaced water as the diluent, osmotic work may have been involved in the first case. Also favoring an osmotic interpretation is the finding that superficial tissues (gill, mantle) showed increased respiration in dilute sea water, whereas muscle showed a decrease.

The stimulated respiration in hypotonic sea water, like that in isotonic NaCl, may involve hydration, since slightly macerated gill showed equally high rates in normal and diluted sea water.

Studies on the red blood cells of fish. Dr. Bruno Kisch.

In this paper investigations on erythrocytes of fish are reported. The hemoglobin content, red blood count, and size of the red blood cells have been measured in different species of Teleostae and Selachians. The investigations were performed in August 1948. The hemoglobin content was determined with a Leitz Haemoglobinometer with colored glass rods as standards for comparison (Sahli's method). The blood count was taken by the usual routine method. The size of the erythrocytes was measured by an ocular micrometer.

Teleosts

Name	No. of animals investigated	Hemoglobin per 1 cc.	Red blood count in 1 mm.^{3}	Diameter in μ of red blood cells
1. Puffer	2	7.35	4,410,000	8.7 : 6.9
2. Scup	1	10.6	3,990,000	8.4 : 6.9
3. Tautog	3	6.7	3,350,000	10.9 : 7.2
4. Porgy	4	13.4	3,170,000	9.8 : 7.2
5. Sea Robin	2	7.1	2,380,000	10.0 : 6.3

Selachians

Name	No. of animals investigated	Hemoglobin per 1 cc.	Red blood count in 1 mm.^{3}	Diameter in μ of red blood cells
1. Mustelus canis	5	3.92	452,000	16.3 : 10.9
2. Carcharinas obsc	1	3.5	540.000	19.0 : 13.5
3. Raja eglant.	2	4.8	320,000	24.3 : 14.2
4. Raja stabilifor.	3	3.6	260,000	21.3 : 14.5

These figures show: Teleosts have a hemoglobin content of the blood 2 to 3 times higher than Selachians and a red blood count which is 5–8 times as high as that of Selachians, but the size of their erythrocytes is about ½ that of the elasmobranch fish.

Among the latter a definite difference has been found between the quick sharks and the slow skates, not so much concerning the hemoglobin content as concerning the red blood cells.

The red blood cells of two Mustelus embryos were much bigger than those of the grown animals, rather rounder than elliptic, and the nuclei showed a chromosome filament. The nuclei were less compact and bigger than those of grown animals.

Among the teleosts, the swordfish showed small blood cells about the size of those of a puffer. The biggest erythrocytes among the bone fish, very similar to those of the elasmobranch fish, were found in a specimen of the plectognate Mola Mola; they were 12.5 : 9.4 in size.

It is the impression of the author that the relation between size and number of red blood cells in fish is only a single instance of a general rule in biology.

Whenever the same function is accomplished in different species, either by a few or by many functioning units, the size of each of the few units is big whereas the size of each of the many units is small. The set-up with the many small units seems to be the more efficient one, the set-up with the few big units the more primitive, less efficient one.

It seems to be similar with the size and number of the glomeruli of the kidney for instance in the murida family and among the Chinoptera as can be judged by the data given by Denzer.

Dr. R. Chambers was kind enough to call my attention to the chromosomes of different species of the family Cyclops where one species (*Cyclops brevispinosus*) has four very big and another (*C. viridis*) twelve very small chromosomes.

Further studies will show how far this rule applies to nerve ganglions and other functioning units of the living beings.

The action of NH_4Cl on the surface membranes of Arbacia eggs. M. J. Kopac.

A new granular layer can be revealed on centrifugation of unfertilized or fertilized Arbacia eggs immersed for several hours in 0.53 M NH_4Cl (3 parts) + sea water (7 parts). This layer, less dense than the cytoplasmic matrix, is believed to contain released lipids (Heilbrunn, *Biol. Bull.*, **71**: 299, 1936). White halves, obtained by centrifugal splitting of unfertilized eggs, when treated with NH_4Cl showed almost as much "lipid" substance as whole eggs. Much of this material, therefore, originates in the cytoplasmic matrix.

Unfertilized Arbacia eggs, after treatment with NH_4Cl, were tested by the oil coalescency method (Kopac, *Cold Spring Harbor Symposia*, **8**: 154, 1940). Following 90 to 120 minutes' immersion, the eggs readily coalesced with oil drops indicating the disappearance of jelly. The coalescency of the treated eggs progressively increased the longer the eggs were exposed to NH_4Cl, thereby indicating a weakening of the vitelline membrane. This was further suggested by the flimsy fertilization membranes that developed after insemination.

Unfertilized eggs, after 4 to 6 hours' exposure to NH_4Cl, were stratified by centrifugation. Oil drops were applied to the surface adjacent to the lipid, matrix, yolk, and pigment zones where coalescence occurred readily. Immediately after coalescence near the matrix zone, a wave of peripheral disintegration encompassed the entire cell surface resulting in complete cytolysis of the cell. On occasion, this effect was obtained at the lipid zone. No cytolysis occurred on coalescence at the pigment zone nor at junction of pigment and yolk zones.

Red halves from centrifuged, NH_4Cl-treated cells sometimes retained a small portion of the matrix. Coalescence with oil drops at this region also induced the peripheral wave of disintegration. No cytolysis occurred when yolk or pigment zones were similarly treated.

A similar peripheral disintegration following coalescence was previously observed in completely denuded eggs. This reaction always occurs when extraneous coats are not present to lend their mechanical support to the underlying protoplasmic surface layer. The absence of peripheral disintegration when oil drops penetrated near the pigment of yolk zones suggests a new role for packed cell inclusions. The packed granules and vacuoles can serve as an inner mechanical support for the protoplasmic surface layer, thus replacing the extraneous coats.

These data suggest that, in addition to releasing bound cytoplasmic lipids, NH_4Cl may alter the surfaces of Arbacia eggs. The principal action, however, appears to be on extraneous coats, e.g., jelly, vitelline membrane, and hyaline layer.

The inhibition of development of Arbacia eggs by NH_4Cl. M. J. Kopac.

Immersion of unfertilized Arbacia eggs in 0.53 M NH_4Cl (3 parts) + sea water (7 parts) for several hours altered, inter alia, the density ratio between cytoplasmic matrix and nucleus. Following centrifugation of treated eggs, the nucleus assumed an equilibrium position near the yolk boundary rather than near the oil cap as in normal eggs.

Nearly all cells immersed in NH_4Cl-sea water up to 4 hours prior to insemination cleaved although there was considerable delay. About 50 per cent of the fertilized eggs divided following 7 hours' exposure. None divided following 13 hours' exposure. Before insemination, the cells were transferred to sea water.

Subsequent development in normal sea water was slow and irregular. Following 2 to 3 hours' exposure to NH_4Cl, 50 per cent of the eggs developed into swimming blastulae. Following 5 to 6 hours' exposure, 10 per cent formed swimming blastulae. Gastrulation was completely inhibited by only 15 minutes' exposure. The blastulae were sluggish and swam in tight circles near the bottom of the vessel.

White and red halves obtained by centrifugal splitting of unfertilized eggs, previously exposed to NH_4Cl for 4 hours, also cleaved following insemination. The red halves cleaved more rapidly than the white halves which contained all the displaceable low density substances. Development was irregular and only a few of the red halves developed beyond the 12- or 16-cell stages.

Fertilization membranes were weak and many ruptured as early as the 2- or 4-cell stage. Double and triple blastulae were common. Frequently, one part of the double blastula was well developed while the other part consisted of disorganized blastomeres which rarely held together. Some of these blastomeres had no hyaline layer as revealed by the oil coalescency method.

Several examples were noted where one blastula, the size of the first blastomere, developed normally while attached to it were 2, 4, or more cells, apparently arising from the second blastomere formed during the first cleavage.

The weakened extraneous coats are probably responsible for this irregular development. The fertilization membranes were weak and these did not elevate from the cell surface more than 2–3 microns. The hyaline layer was variable in thickness even during the first or second cleavages. Later, it frequently became so weak that bizarre aggregates of cells were produced instead of organized blastulae.

The nature of the hemolytic effect of silver. MARIAN E. LEFEVRE AND M. H. JACOBS.

The hemolysis of certain fish erythrocytes by an impurity in some brands of C. P. NaCl, shown by Ball to be silver, has been further studied, using chiefly the erythrocytes of two sensitive species, the mackerel and the cunner. It has been determined that the mechanism of this hemolytic effect is an induced permeability of the erythrocytes to cations resulting in a swelling of the cells by the operation of the Donnan equilibrium. The evidence is: (1) that immediate and extensive shrinkage of the cells occurs in sucrose made isosmotic with the blood and containing a trace of calcium, (2) that swelling and hemolysis are rapid in NaCl of any strength, (3) that on the addition of concentrated NaCl to a suspension of cells in an appropriate mixture of NaCl and sucrose there is an immediate shrinkage, quickly followed by a return to more than the original volume, (4) that hemolysis in NaCl is prevented by the addition of relatively low concentrations of sucrose, provided that Ca is present, and, (5) that under properly chosen conditions the rate of hemolysis after treatment with silver shows a minimum at a pH near the isoelectric point of hemoglobin.

In the course of this work it was noted that the erythrocytes of the two species of fish in question, and some others, undergo hemolysis within an hour or less in pure isosmotic sucrose solutions. As in the case of the much less striking injury of mammalian erythrocytes in the non-electrolyte solutions studied by Wildbrandt, hemolysis of the fish erythrocytes was only feebly opposed by NaCl but very effectively by low concentrations of $CaCl_2$, $BaCl_2$, or $MgCl_2$.

It was also noted that the well-washed erythrocytes of several species of fish, unlike mammalian erythrocytes, may fail to hemolyze within several hours in a pure isosmotic solution of NH_4Cl. On the addition of a trace of bicarbonate, however, hemolysis in the same solution readily occurs.

Reversible sphering of erythrocytes. WARNER E. LOVE AND M. H. JACOBS.

The sphering action on human erythrocytes of sodium taurocholate and the fact that it is favored by alkalinity are well known. It has apparently not previously been reported, however, that sphering of taurocholate treated cells can be produced and reversed at will many times in succession merely by appropriate changes in the pH of the surrounding medium.

A convenient way of showing this effect is to suspend well washed human erythrocytes in an isotonic NaCl solution buffered about neutrality with phosphate and lightly colored with phenol red, which in low concentrations has little effect on the cells. With a little NaOH the indicator is now made distinctly red and taurocholate is added until sphering just occurs. On the addition of HCl until the indicator becomes yellow the cells immediately resume their biconcave shape. In the absence of phenol red the behavior of the cells themselves serves as a sensitive indicator of pH changes.

Alkali and acid may now be added alternately as often as desired. We have in this way produced sphering and its reversal 15 times in succession. If only enough taurocholate be used to cause sphering, the cell may remain in good condition and be capable of showing reversal of sphering for at least 24 hours. Several washings of the taurocholate-treated cells with isotonic NaCl do not destroy their ability to show the behavior just described. Under favorable conditions a pH change of 0.5 unit or less is sufficient to reverse the taurocholate effect, but even much larger changes have little influence on the sphering produced by sodium cetyl sulfate or sodium cetyl phosphate.

Observations on the hemolytic effect of sodium dodecyl sulfate. LOIS H. LOVE AND M. H. JACOBS.

The hemolysis of human erythrocytes by sodium dodecyl sulfate has several unusual aspects. The first is the effect of temperature on the rate of the process. At low temperatures with some concentrations complete hemolysis occurs within a few seconds, but as the temperature is increased, a point is reached where the process suddenly becomes very slow. Under some conditions a rise of temperature of 1° C. may change the time for complete hemolysis from less than a minute to more than an hour. With a further rise of temperature the rate of hemolysis may again become more rapid.

In the temperature range of retardation two phases of hemolysis can be demonstrated, some of the cells hemolyzing immediately, the remainder very slowly. The slowly hemolyzing cells, however, show a normal susceptibility to the hemolytic action of hypotonic solutions.

The second peculiarity of hemolysis by sodium dodecyl sulfate is that the addition of lecithin causes a remarkable acceleration of the process instead of the retardation reported in the case of certain other hemolytic agents. For example, in one experiment 80 per cent hemolysis by sodium dodecyl sulfate alone required 160 minutes; in the presence of approximately $50\,\gamma$ of lecithin alone no hemolysis occurred in 180 minutes. But in a mixture of the two the time for 80 per cent hemolysis was 16 minutes. By a proper choice of conditions even an apparently non-hemolytic concentration of sodium dodecyl sulfate can be made hemolytic by addition of a non-hemolytic concentration of lecithin.

Effects of hypertonic solutions on Nereis eggs. W. J. V. OSTERHOUT.

Unfertilized eggs of *Nereis limbata* placed in 1.4 M $MgSO_4$ or in 1.5 M dextrose in sea water lose water rapidly and appear collapsed with abnormal shapes. If after a few minutes they are replaced in sea water, they soon become normal in size and appearance. If sperm is promptly added many eggs extrude jelly, segment and develop trochophores.

If the unfertilized eggs are placed in 2.7 M $MgSO_4$ in sea water, they collapse at once, but in the course of time $MgSO_4$ enters the eggs sufficiently to enable them to resume more nearly normal shape. The same thing happens in 2.8 M dextrose in sea water but here the penetration is much more rapid.

When the unfertilized eggs are exposed for a short period to 2.7 M $MgSO_4$ in sea water and are returned to sea water they swell rapidly and develop a clear zone just beneath the vitelline membrane. Such eggs cannot extrude jelly nor segment nor produce trochophores when sperm is added.

Solubility of the vitelline membrane of Nereis eggs. W. J. V. OSTERHOUT.

The vitelline membrane of Nereis resists acid and alkali and all other reagents hitherto tried. But it dissolves quickly in a mixture of sodium dodecyl and tetradecyl sulfate. When unfertilized eggs are placed in 1 per cent of this reagent in sea water the egg swells and soon shows a protrusion at one point as though the tough vitelline membrane were yielding to internal pressure.

The membrane covering the end of the protrusion dissolves and this action spreads to surrounding regions and in a few minutes the membrane completely disappears. A similar process takes place in segmenting eggs.

The dissolving of the vitelline membrane is more rapid and more easily observed if the eggs are first killed by placing them for a few minutes in 2.7 M $MgSO_4$ in sea water and then leaving them for a short time in sea water before they go into the dissolving reagent. In sea water they swell and acquire a clear zone inside the vitelline membrane. In the reagent splits appear at several places in the membrane and at these spots the membrane dissolves and this action spreads to neighboring regions so that the membrane completely disappears. This process is accompanied by alterations in the appearance of the protoplasm.

Experiments on chloroplasts and on photosynthesis. W. J. V. OSTERHOUT.

The chloroplasts of certain marine and fresh water algae and of some other plants show very interesting reactions when the cells are bathed in a saturated solution of hexylresorcinol. The chloroplasts shrink and clump together and in many cases form a more or less regular network. This continues to contract and may eventually form a single compact mass occupying only a small portion of the cell.

There is great loss of water from the chloroplast and the minute drops of chlorophyll (grana) originally present in the chloroplast are evidently brought closer together and it seems possible that they may fuse to some extent.

Chloroplasts exposed to "Sodium Lorol Sulfate" (which may be called S. L. S. for convenience) also show great contraction. This reagent is a mixture of sodium dodecyl and tetradecyl sulfate. When cells of *Spirogyra* are placed in 1 per cent S. L. S. in distilled water, the chloroplast contracts and eventually breaks up into small masses. The green color soon spreads uniformly throughout the cell as if in aqueous solution but is unable to pass out through the cellulose wall. Cells in this condition show no photosynthesis when well washed and placed in a solution of sodium bicarbonate with a trace of phenolphthalein as indicator. But cells subjected for 2 minutes to 0.01 per cent S. L. S. in which the chloroplast is still intact show photosynthesis though slightly delayed as compared with the control. When the cells are exposed for 8 minutes to the same reagent, the cells at first appear not to be seriously affected, but on washing thoroughly and transferring them to the bicarbonate solution containing phenolphthalein, the injury progresses until the chloroplasts are affected. Such cells show no photosynthesis within 1 hour or longer. The control cells show rapid photosynthesis.

A new peritrich from Woods Hole. M. A. RUDZINSKA.

Pachystomus Olistus (cf. Abstract Section *Biological Bulletin,* **93**: 2, 1947).

Inter-myotome connections in early embryos of Mustelis canis. LOIS E. TEWINKEL.

Embryos of the smooth dogfish, *Mustelis canis,* begin to flex rhythmically from side to side at an approximate length of 3.5 mm. (20–23 somites). Such motion, observed in a number of elasmobranch embryos and shown by Wintrebert to be aneural, is exhibited for some time prior to the initiation of the heart beat, which, in the Mustelis embryos studied, occurred at the 5.5–6 mm. stage.

Sections in the three usual planes show that the antero-posterior orientation of myoblasts has occurred in at least the anterior two-thirds of the somites in 3.5–4 mm. embryos. Cell boundaries are virtually impossible to distinguish but preliminary studies indicate that myoblasts of a given somite come into contact with those of immediately adjacent somites so that there is an interdigitation of their tapering ends. Fibrillar structures seen in the cells are identified tentatively as early myofibrillae. In general they are homogeneous but occasional faint signs of beading suggest the development of cross striations. In 5.8 and 8 mm. embryos, myoblasts of one myotome seem to be directly connected to those of neighboring myotomes at a more constricted area. Distinctly striated myofibrillae, which are now present, appear to be joined to those in cells of adjacent myotomes by means of fine light-staining homogeneous fibrils which extend across the inter-myotome bridge.

At the 35 mm. stage, additional bundles of striated muscle cells have differentiated apparently from mesenchyme cells between the myotome bands and the notochord, but these bundles are separated inter-segmentally by numerous mesenchyme cells. The contrast, therefore, between the longitudinally connected primary myotome bands and the segmented muscle bundles which develop later suggests that there may well be some relationship between myotome bridges and the rhythmic flexion of the early embryo.

Properties of the surface coat in embryos of Fundulus heteroclitus. J. P. TRINKAUS.

The eggs of Fundulus possess a surface layer similar to the surface coat of amphibian eggs. This coat constitutes the outer layer of the egg in all stages examined (immature, mature eggs, and developmental stages through blastopore closure). Microdissection demonstrates it to be an elastic gel, non-adhesive on the outer surface, sticky and less viscous at deeper levels. It solates in Ca-free SW and upon mechanical agitation.

A wound in the coat covering the yolk first widens and then closes in 2–5 minutes (even though yolk is exuded throughout the process). During wound closure, radiating folds appear in the coat. When carbon particles are placed on opposite edges of a closing wound they move toward each other, indicating that wound closure is not due to the formation of a new surface, but to the expansion of an already present, elastic coat. Closure of a wound in the yolk coat near the marginal periblast apparently exerts tension, which not only stretches adjacent cells greatly, but also pulls a large sector of blastoderm toward the wounded area. A wound in the blastoderm itself initiates similar processes of wound healing, resulting in radial stretching of epiblast cells toward the wound closure.

Carbon marking of normal cleavage and gastrula stages suggests a spreading of the surface layer in both cleavage and epiboly. At closure of blastopore of normal embryos, the cells at the blastopore lips elongate greatly, as if they are being pulled toward the point of blastopore closure. At an earlier stage (Oppenheimer, '14), this phenomenon can be simulated and blastopore closure hastened, if the yolk coat is wounded at the point of future closure of blastopore.

These preliminary studies suggest that the surface coat of the Fundulus egg, because of its remarkable elastic properties, plays a unifying and perhaps a causal role in gastrulation movements.

Fertilizin of Nereis limbata. ALBERT TYLER.

The fertilizins of eggs of sea-urchins and other marine animals are known to comprise the material of the gelatinous coat of the unfertilized egg. Since, in Nereis, a gelatinous coat forms after fertilization it is of interest to learn whether or not the material of this coat is fertilizin and to determine some of its chemical properties. Using the alkaline saline method of Costello (1945) for removal of the membrane, and extrusion and dissolution of the Nereis egg jelly, large quantities of this material were prepared with very little injury to the eggs. When added to sperm, under proper conditions of pH (ca. 9) the preparations gave strong agglutination, the titers being considerably greater than those given by ordinary egg water prepared from unfertilized egg in the same time interval. While definitive proof is yet to be obtained, various tests favor the view that the jelly material is fertilizin and that that present in ordinary egg water is similar material that has diffused through the membrane in non-gelatinous form. The active material is found to be non-dialyzable, fairly heat stable and precipitable by 60 per cent alcohol. It has a nitrogen content of about 5 per cent and contains at least 18 per cent reducing sugar. From a total of approximately 20 cc. of eggs that have been obtained so far in the present season, the yield of alcohol precipitable material amounts to 300 mg., most of which is available for further analysis.

Mating types and conjugation of four different races of Paramecium calkinsi and the effect of x-rays on the mating reaction. RALPH WICHTERMAN.

Four different races of *Paramecium calkinsi* have been cultivated up to two years in a medium consisting of 2 parts of lettuce infusion and one part of filtered sea-water. Opposite mating types I and II (Yale races) are unimicronucleate; the remaining strains here designated as IIa and IIb are unimicronucleate and bimicronucleate respectively as shown in Hematoxylin and Feulgen preparations. Micronuclei in all races are small and average only 2 μ. Races IIa and bimicronucleate IIb readily mate and conjugate with unimicronucleate type I. All races appear to be as stable as *P. bursaria* since no change in mating type has occurred during the period in culture. In all cases, the mating reaction occurs with the formation of clumps of a 100 or more specimens any time of day, followed by conjugation. Cultures containing *P. calkinsi* have, at approximately 24° C., a pH range of 6.5 at time of inoculation to pH 7.8 in older cultures. Greatest numbers of paramecia reactive for mating are found in cultures with a pH of 7.3 usually on the 7th day after inoculation.

P. calkinsi is much more sensitive to x-rays than *P. bursaria*. When opposite mating types are irradiated with 100,000 r, 200,000 r and 300,000 r and mixed, the mating reaction occurs but the clumps thus formed are progressively smaller than the controls. Clumps then break down leaving only single specimens. However, mating types irradiated with 100,000 r and mixed will, 24–48 hours later, demonstrate pairs in conjugation. Those irradiated with 200,000 r and 300,000 r will demonstrate the mating reaction but do not enter into conjugation with the exception of some survivors of 200,000 r dosage. When irradiated with 400,000 r and mixed, the slowly moving specimens do not show the mating reaction but die within a few hours. All unirradiated members of one sex type show the mating reaction with specimens of opposite type irradiated up to 400,000 r. Only those irradiated with 100,000 r and some survivors of 200,000 r will conjugate with unirradiated specimens of opposite type. Unirradiated specimens of one sex type will not mate or conjugate with irradiation-killed specimens of opposite sex type.

The hydrogen-ion concentration in the cultivation and growth of eight species of Paramecium. RALPH WICHTERMAN.

A study was made of the pH changes occurring daily in 36 cultures of races of eight fresh and brackish-water species of Paramecium. Original cultures have been maintained continuously for 1–13 years in covered 250 ml. flasks with little if any variation in culture technique. *P. aurelia, P. caudatum, P. multimicronucleatum* and *P. trichium* have been cultivated in hay medium consisting of 1½ gm. of hay and 210 ml. of boiled distilled water which prior to inoculation had a pH of 6.2; *P. bursaria* and *P. polycaryum* in lettuce medium consisting of 1½ gm. desiccated lettuce to 1 liter of boiled distilled water which was filtered then autoclaved and had a pH of 5.0 prior to inoculation; *P. calkinsi* and *P. woodruffi* on lettuce-sea-water medium with a ratio of 2 : 1 respectively and a pH of 6.5 before inoculation.

All media were prepared on one day and inoculated the following day with approximately 20 ml. from a rich culture containing paramecia and bacteria. Daily pH determinations, which in the course of the investigation totalled over 500, were made of the medium prior to inoculation, through the period of maximal growth and gradual decline of the population at about 24° C. with a Cambridge electronic pH meter having a sensitivity of 0.02 pH unit. Buffers were not added to cultures. Results are briefly summarized in the table.

Species	Medium and pH before inoculation	pH range of medium in cultures	Optimum range in which growth occurred and yielded greatest concentration of paramecia
P. aurelia	Hay: 6.2	6.2–7.3	7.0–7.2
P. caudatum	Hay: 6.2	6.2–7.2	6.9–7.1
P. multimicronucleatum	Hay: 6.2	6.2–7.5	6.5–7.0
P. trichium	Hay: 6.2	6.2–7.1	6.7–7.1
P. bursaria	Lettuce: 5.0	5.0–7.4	7.1–7.3
P. polycaryum	Lettuce: 5.0	5.0–7.5	6.9–7.3
P. calkinsi	Lettuce-sea H_2O: 6.5	6.5–7.8	7.1–7.4
P. woodruffi	Lettuce-sea H_2O: 6.5	6.5–7.5	7.0–7.5

The cage hypothesis and a common feature of X-ray diffraction studies of crystalline proteins. DOROTHY WRINCH.[1]

It is well recognized that X-ray diffraction data obtained from protein crystals constitute—potentially at least—crucial tests for any proposed theory of the atomic patterns of proteins.

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[1] Smith College.

Fundamental difficulties however arise in using these data for this purpose in any detailed manner in that (1) a protein unit is in general an array of molecules, (2) a protein molecule comprises a skeleton plus a complement of substituents in most cases incompletely characterized and in all cases unknown as regards their spatial pattern, (3) protein crystals contain large numbers of "foreign" molecules and ions, (4) the diffraction patterns to be expected from vast arrays of atoms present scientific and technical problems of considerable complexity (Wrinch, Fourier transforms and structure factors, Am. Soc. for X-Ray and Electron Diffraction, 1946).

In a hypothesis formulated in 1936 (Wrinch, *Proc. Roy. Soc. London* **A161**: 505, 1937), protein molecules were formulated as cage structures, space-enclosing networks of multiply-connected α-levo amino acid backbones, with R-substituents emerging from C_α atoms. Naturally it has, as yet, not proved possible to allocate particular substituents to particular C_α sites, nor even to formulate the numbers or the spatial patterns of the molecules in various protein particles. The fact that this hypothesis gives a skeletal structure which is cubic leads to a picture of protein particles with molecular patterns in which the orientations of the skeletons are structurally related, and thus to the possibility that the constructional principle common to proteins may prove, in some measure, recognizable in diffraction patterns, notwithstanding the fact that the skeletal atoms may constitute a third or less of the atoms in the crystal.

This communication reports the fact that the series of cage structures, C_1, C_2, . . . C_n, . . . have one common feature in their diffraction patterns. The interaction of one pair of antipodal tetrahedral faces of the C_n structure yields maxima in diffraction space, at distances 8 *an* (1, 1/2, 1/3, . . . 1/*n*, . . .) A from the origin, where *a*—say ∽ 1.5A—is the mean of the N–C_α, C_α–C, C–N bond lengths. Thus, for the whole series, these interactions produce a maximum at ∽ 12A. The remaining three pairs of antipodal faces modify the maximum but slightly, moving them from ~ 12A to ~ 10A in C_1, from ~ 12A to ∽ 11.2A in C_2, from ∽ 12A to ∽ 11.6A in C_3, and so on. When the closely packed substituents on the cage faces are taken into account, these maxima may move to slightly shorter spacings (loc. cit., p. 55).

It is well known that crystalline proteins as a class yield strong spacings in the neighborhood of 10–11.5A. It may therefore be claimed that the cage hypothesis meets the first challenge of the X-ray data in that it predicts one or more of a series of structures, all of which have pairs of antipodal faces which, with the remaining faces, produce high intensities at distances of this order of magnitude. It is pertinent to notice that the maxima derived from the predicted skeletons lie in octettes at the corners of a cube and that in the case of tobacco mosaic virus strong spacings at ∽ 11.3A, close to cube corners, have been recorded (Bernal and Fankuchen, *J. Gen. Physiol.*, **25**: 111, 1941).

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REPORT ON THE LALOR FELLOWSHIP RESEARCH

Phosphagen in annelids (Polychaeta). Ernest Baldwin * and Warren H. Yudkin.

Kutscher and his school came to the conclusion, upheld by Andrew Hunter, that the creatine which is characteristic of vertebrate muscle is replaced by arginine in the muscles of invertebrates. The discovery of creatine phosphate in the muscle of vertebrates by Eggleton and Eggleton (*Biochem. J.*, **21**: 185, 1927) and Fiske and Subbarow (*J. Biol. Chem.*, **81**: 629, 1929) was shortly followed by that of arginine phosphate in invertebrates by Meyerhof and Lohmann (*Biochem. Ztschr.*, **196**: 22, 49, 1928). Comparative investigations by the Eggletons (*J. Physiol.*, **65**: 15, 1928) and by Meyerhof (*Arch. di Sci. Biol.*, **12**: 536, 1928) supported the broad principle of alternative occurrence. The wider comparative studies of Needham, Needham, Baldwin, and J. Yudkin (*Proc. Roy. Soc. London B*, **110**: 260, 1932) upheld this general principle, but with certain notable exceptions: both phosphagens were found side by side in the body muscles of *Balanoglossus* and in the jaw muscles of an echinoid. These results provided chemical support for the echinoderm-enteropneust theory of vertebrate ancestry proposed by Bateson on morphological grounds. Our present investigation has demonstrated that the enteropneust, *Saccoglossus*, contains only the single phophagen, creatine phosphate.

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* Lalor Fellow.

One of the objects of our present study was to investigate the phosphagen of annelids more carefully than has hitherto been done for, while Meyerhof and Needham, Needham, Baldwin and J. Yudkin alike had demonstrated the presence in polychaetes and gephyreans of a labile substance having the general properties of arginine phosphate, Arnold and Luck (*J. Biol. Chem.*, **99**: 677, 1933) were unable to find evidence for the presence of arginine itself in any of the marine worms they studied, though arginine was found in the terrestrial oligochaete *Lumbricus* and has in fact been isolated from this form by Kutscher and Ackermann (*Ztschr. physiol. Chem.* **199**: 266, 1931). Moreover, in the original experiments of Needham, Needham, Baldwin, and J. Yudkin the phosphagen of *Nereis diversicolor* showed somewhat anomalous behavior which was not further investigated at that time. Recently, Greenwald (*J. Biol. Chem.*, **162**: 239, 1941), employing Jaffe's reaction, demonstrated the presence of chromogenic material indicative of creatine in the testes of several annelids.

In the present investigation experiments were done on the atypical behavior of *Neanthes* phosphagen upon hydrolysis in the presence and the absence of the molybdate ion. These experiments showed the presence in this annelid of a labile phosphate compound having the properties of creatine phosphate, together with a phosphagen similar in behavior to arginine phosphate, which for the present we propose to call the "annelid phosphagen." Studies of other polychaetous annelids have now shown that the apparent creatine phosphate and the annelid phosphagen may occur singly or together. From Table I we may generalize concerning the species examined: All the free swimming forms contain creatine phosphate, sometimes together with the annelid phosphagen; all the sedentary forms, with the exception of *Chaetopterus*, contain the annelid phosphagen, sometimes together with creatine phosphate.

TABLE I

Occurrence of phosphagen in polychaetes

The annelid phosphagen similar to arginine phosphate is signified by AP. CP indicates the phosphagen behaving like creatine phosphate.

	AP	CP
Free Swimming Forms (Errantia)		
Orbinia (*Aricia*)	−	+
Diopatra	−	+
Lumbrineris (*Lumbrinereis*)	−	+
Arabella	−	+
Glycera	−	+
Neanthes (*Nereis*)	+	+
Lepidometria	+	+
Sthenelais	+	+
Sedentary Forms (Sedentaria)		
Chaetopterus	−	+
Cistenides	+	+
Amphitrite	+	−
Pista	+	−
Enoplobranchus	+	−
Cirratulus	+	−
Maldane	+	−
Clymenella	+	−

These newer observations show that Needham, Needham, Baldwin, and J. Yudkin's former conclusions must be seriously modified. Whether they must actually be abandoned will depend on the results of further, thoroughgoing investigations of other annelid groups and of more representatives of other invertebrate phyla.

We are pleased to acknowledge our indebtedness to Dr. Frank A. Brown, Jr., for procuring and identifying most of the annelids used in this study.

The mechanism of interaction of inhibitors with human plasma cholinesterase. AVRAM GOLDSTEIN.*

The carbamic esters prostigmine, physostigmine, and carbaminoylcholine inhibit purified human plasma cholinesterase activity as determined by addition of acetylcholine to an enzyme-inhibitor mixture. During a period of two hours after addition of substrate there is a progressive relief of inhibition which is not the result of destruction of the inhibitor. Thus acetylcholine *slowly* displaces these inhibitors from combination with the enzyme. The dissociation that occurs on dilution of an enzyme-inhibitor mixture is also slow, requiring two to three hours for completon. These compounds are reversible inhibitors (by dialysis). Determination of the nature of inhibition by varying substrate concentration by the method of Lineweaver and Burk (*J. A. C. S.*, **56**: 658, 1934) reveals an apparent non-competitive inhibition, i.e., in a twenty minute period of determination no amount of substrate can reverse the combination. If, however, substrate is given full access to the enzyme (by its addition simultaneously with inhibitor) completely competitive curves are found. . . . In contrast to the above, choline, acetyl-b-methyl choline, procaine, methylene blue and other reversible inhibitors are competitive in the varying substrate experiments, but their displacement by substrate proceeds very rapidly. . . . A curious paradox is presented by mercuric ion (chloride) which inhibits irreversibly (by dialysis) yet gives *competitive*, or partially competitive curves in the Lineweaver-Burk method—i.e., appears to be displaced immediately by acetylcholine. . . . It may be inferred that while the carbamic esters described combine at the substrate-active center, mercuric ion does not; but that combination of substrate changes the affinity of the latter for the protein moiety of the enzyme. It is suggested that "competitive inhibition" need not imply competition with substrate for the same site of attachment to the enzyme.

Complexes of hemocyanin and of hemorythrin with small ions. I. M. KLOTZ * AND F. TIETZE.

Previous investigations (*Biol. Bull.*, **94**: 40, 1948) have demonstrated that the serum of *Limulus polyphemus* is capable of combining with small molecules other than those involved in its respiratory function. To establish that the binding is by the hemocyanin component of the serum, this protein has been isolated, and its ability to form complexes with several organic ions has been examined.

Quantitative estimates of the binding of methyl orange by isoelectric hemocyanin have been obtained from equilibrium dialysis experiments. At dye concentrations as high as 7×10^{-5} molar, an average of about 0.1 mole of anion is bound by each unit (of 37,000 molecular weight) of the hemocyanin. This is of the same order of magnitude as was found for an equivalent quantity of this protein in the serum.

Qualitative evidence of the binding of several other ions by hemocyanin has been obtained from spectrophotometric observations.Thus the spectrum of a mixture of Orange II and the protein differs from the sum of those for the pure components by a shift of 150 Å in the region of the dye's peak near 4850Å, as well as by an increased absorption in the 5800 Å region of the chromoprotein. With salicylate ion, in turn, which has no spectrum in the visible region, only an increased absorption in the 5800 Å band is observed. A small change in the absorption of solutions of the cationic dye, methylene blue, in the presence of hemocyanin has been found also, but its interpretation is doubtful, since the dye is aggregated, even at these low concentrations, and small shifts on addition of protein might be due merely to changes in the dielectric properties of the medium.

Hemerythrin, isolated from a sample of serum of *Phascolosoma gouldi* kindly given to us by Dr. Ernest Baldwin, shows even more pronounced evidence of complex formation with small ions. Thus in dialysis experiments with the anionic dye, Orange II, an average of 2.5 moles of ion is bound, by each unit of 100,000 molecular weight, at a free dye concentration of slightly less than 1×10^{-4} molar. (The unit of 100,000 molecular weight was chosen arbitrarily, for purposes of comparison, since adequate data on this protein are not available.) Similarly, in spectrophotometric studies, the absorption peak at 5000 Å is shifted over 500 Å to shorter wave-lengths, and the optical density is doubled, on the addition of thiocyanate to the protein. Similar effects are obtained with other known iron-complexing agents such as cyanide or hydroxylamine hydrochloride.

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* Lalor Fellow.

Thus it seems probable that many of the respiratory pigments can act as ion-transport agents, in addition to their primary function as oxygen carriers.

Further studies on the mechanism of alloxan action; the reaction of alloxan with sulfhydryl groups; the glutathione content of islet tissue. ARNOLD LAZAROW.*

It has been suggested that alloxan produces diabetes because of inactivation of essential sulfhydryl enzymes and that the selectivity of alloxan for the beta cells may be due to a low glutathione content (Lazarow, A., *Proc. Soc. Exp. Biol. and Med.*, **61**: 441, 1946).

Alloxan has been shown to react with glutathione and protein at pH 7.4 to give a new compound with an absorption spectra maximum at 305 mμ [Lazarow et al., *Science* (in press)]. When p-Cl-Hg benzoate is added to a glutathione solution prior to the addition of alloxan, no "305" is formed. Since p-Cl-Hg benzoate is a specific sulfhydryl reagent, the failure of formation of "305" indicates that alloxan is reacting with the sulfhydryl group of glutathione. These in vitro studies suggest the possibility of protecting animals against alloxan diabetes by injecting p-Cl-Hg benzoate prior to a diabetogenic dose of alloxan. Following the disappearance of the injected alloxan (spontaneous decomposition occurs at pH 7.4 with a half-life of 1 minute at 37° C.), it may be possible to reactivate the sulfhydryl groups by the addition of BAL, which removes the mercury compound.

Determinations have been carried out on the glutathione content of the islet tissue of fish. The ferricyanide method of Mason was adapted for micro-analysis in final volumes of 0.5 cc. The principal islet of the goosefish (*Lophius piscatorius*) contains 27–58 mgs. glutathione/100 gms. tissue (average glutathione of 9 fish was 50 mgs./100 gms.). This value is lower than the glutathione content of liver or kidney, and greater than that of muscle. Although the islet glutathione values represent the average glutathione content of the alpha, beta, and gamma cells, it may be possible to determine the glutathione content of the beta cells by indirect means [using the glutathione content of alloxan diabetic fish (alpha and gamma cells) and the percentage composition of cell types].

The insulin content of the islet tissue of alloxan diabetic fish. ARNOLD LAZAROW * AND JACK BERMAN.

Diabetes was produced in the toadfish by injecting alloxan subcutaneously in doses of 600 mgs./kg. body weight. Serial blood sugar determinations were carried out by the Folin-Malmros microblood sugar method. After the removal of the islet tissue, it was homogenized in ice-cold saline and serials dilutions were prepared. Doses, ranging from .05 to 1.0 mgs. of islet tissue, were injected into mice which had been starved for 24 hours (three animals were used for each dilution). Blood samples were drawn from the tail vein at 0 and 30 minutes after injection.

Using the islet tissue of normal fish, the percentage drop in blood sugar was found to be proporportional to the log of the injected islet dose. When liver homogenates or saline were injected into mice, under similar conditions, they resulted in an elevation of blood sugar.

The islet tissue of alloxan diabetic fish (sugars greater than 400 mgs./100 cc.), assayed 48 hours after the injection of alloxan, showed considerable quantities of a blood sugar lowering factor (insulin). (The injection of 0.1 mg. or less of islet tissue per mouse produced a 25–40 per cent drop in blood sugar.)

Although the limited number of analyses carried out so far do not permit accurate comparison between the insulin content of the islets of normal and diabetic fish, they do indicate that both may be of the same order of magnitude.

The presence of sizable quantities of insulin in the islet tissue of alloxan diabetic fish raises the question as to whether there may be a dissociation between insulin storage and insulin secretion, or whether factors other than insulin deficiency may play a role in the etiology of the hyperglycemia which persists after alloxan injection.

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* Lalor Fellow.

A New Concept of the Action of Dicumarol. JOSEPH LEIN.[1] *

All previous work with Dicumarol has tended to confirm the view that the drug acts by decreasing the prothrombin content of the blood by preventing its synthesis in the liver. The evidence for this view has been obtained by determining clotting times of Dicumarol treated plasma using a protein thromboplastic agent. Such plasma shows a markedly increased clotting time.

In addition to protein thromboplastic agents there is also a lipid thromboplastic agent which has a considerably lower clotting activity than the protein with normal plasma. Experiments were carried out in which the clotting times of rabbits treated with Dicumarol were determined with the lipid and protein thromboplastic agents.

Three day treatment of rabbits with 10 mg. of Dicumarol per kg. per day caused a marked increase in the clotting time of rabbit plasma using the protein thromboplastic agent, while the clotting time obtained with the lipid agent increased but slightly. Thus, in one case the protein agent did not clot the plasma in over three hours while the lipid clotted it in five minutes. Comparison of the protein thromboplastic agent clotting times with control clotting times indicates further that the protein thromboplastic agent acts as an inhibitor of the clotting of Dicumarol plasma. Addition of highly purified prothrombin brings the relationships to those found in normal plasma.

Since reduction of prothrombin concentration of plasma by physical methods of dilution or absorption on aluminum hydroxide does not reverse the relative thromboplastic activities of the lipid and protein agents, it is believed that Dicumarol treatment does not prevent the synthesis of prothrombin by the liver but causes the synthesis of an altered prothrombin. This altered prothrombin is not converted to thrombin by the action of a protein thromboplastic agent but is converted by the action of a lipid agent.

Enzyme localization in the giant nerve fiber of the squid. B. LIBET.[2] *

Last summer it was found that the enzyme ATP-ase is localized almost exclusively in the sheath of the giant axon, with practically none in the axoplasm. This sheath ATP-ase showed a rate of activity even greater than that of squid muscle, and also certain other similarities to muscle ATP-ase. A further analysis of the properties of this nerve ATP-ase shows the following: (a) The rate of activity is increased by increasing concentration of either Ca or Mg ions, up to a maximum. (b) The maximum activity is greater with Mg and occurs at a lower concentration (about 0.003 M for Mg is almost maximal; about 0.03 M for Ca). (c) Mg stimulates at all K ion concentrations (up to 0.58 M), unlike the situation in muscle, where it inhibits at high K. (d) When Ca and Mg are added together there is antagonism, but in the reverse direction from that reported for muscle ATP-ase; i.e., Ca inhibits the Mg activation in nerve.

Neither sheath nor axoplasm splits any other phosphate esters tested at an appreciable rate. β-glycerophosphate is split at a very low rate even at pH 9.1 in the presence of Mg and glycine, so that "alkaline phosphatase" is very low. ATP-ase seems to be the dominant phosphatase present.

An attempt was made to localize further the ATP-ase within the sheath itself by separating the axolemma from the rest of the sheath, but this has yielded inconclusive results thus far. Since the sheath of the giant nerve fiber contains a high percentage of connective tissue (C. T.), ATP-ase was determined in muscle-free C. T. samples taken from the mantle where the latter faces the dorsal side of the pen of the squid.[3] This muscle-free C. T. showed an ATP-ase activity of about 7 μg. P/mg. wet wt./30 min. at 27° C. (compared to a usual figure of about 15–20 for the axon sheath); it is activated by Ca^{++} and does not split β-glycerophosphate or hexose-di-phosphate appreciably. If one could assume that the C. T. of the axon sheath has an activity identical with that of this muscle-free C. T., considerable activity would still be left for the non-C. T. elements. An attempt at further localization will be made histochemically by

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[1] Department of Zoology, Syracuse University, Syracuse, New York.

[2] This work was also supported in part by the Navy Nervous System Research contract with the University of Chicago.

[3] I am indebted to Dr. Magnus Olson for identifying the muscle-free C. T. Only a trace of muscle, that in small blood vessel walls, was found.

Dr. G. Gomori. Obviously results obtained by others on the distribution of other enzymes between sheath and axoplasm in the giant nerve fiber must also be re-examined in the light of these findings.

The choline acetylase and choline esterase content of some invertebrate tissues. HAROLD PERSKY * AND MARCIA GOLD.

The hypothesis that acetylcholine plays the primary role in the generation of the nervous impulse requires that the enzymes synthesizing and decomposing acetylcholine (ACh) be present in significant amounts in all animals possessing a differentiated nervous system. In order to test this premise, the choline acetylase (ChAc) and choline esterase (ChE) content of representatives of the three phyla with the most primitive nervous systems were determined. *Tubularia crocea* (Coelenterate), *Euplanaria maculata* (Platyhelminthes) and *Neanthes virens* (Annelida) were the organisms studied. The entire organism was employed in the first two animals because of the difficulties associated with the dissection of the nervous system in these species. The first segment of *Neanthes* was used because it contains the "brain." The methods of assay for both enzymes were essentially those of Nachmansohn. All the results are expressed as milligrams acetylcholine formed or split per hour per hundred milligrams protein nitrogen (Q). The data is summarized in the following table.

Species	Q_{ChAc}[1]	Q_{ChE}[1]	Q_{ChE}/Q_{ChAc}	$\frac{\text{Protein nitrogen}}{\text{Wet weight}} \times 10^2$
Tubularia crocea	0.03(0.002)	180(1.3)	∞	0.75
Euplanaria maculata	23. (0.25)	2000(20.)	85	1.1
Neanthes virens	20. (0.3)	280(4.2)	14	1.5

[1] Figures in parentheses are the Q values expressed per 100 mg. wet weight. All data at 20° C.

It is apparent from the data that choline esterase is present in all three species in amounts equal or greater to that in rat brain (Q_{ChE} of 210). Since the ChE of these two species is the nerve-muscle type (Nachmansohn), the concentration of ChE in the nervous tissue of these two species may be from 10^2 to 10^4 times that of vertebrate brain. The problem of why such large amounts of ChE are present in such relatively poorly developed nervous systems requires greater study.

The choline acetylase determinations posed greater technical problems than the ChE assays. Acetylcholine synthesis has not yet been shown to be proportional to enzyme content in vertebrate brain or nerve or even highly purified choline acetylase. However, by employing low tissue concentrations, short incubation periods and low temperatures, first order responses between enzyme activity and enzyme content were obtained in the case of *Euplanaria* and *Neanthes*. No such proportionality was obtained in the case of *Tubularia*. In all cases, the absolute amount of acetylcholine synthesized was small. In spite of these shortcomings, the ChAc content of *Euplanaria* and *Neanthes* exceeded vertebrate brain. *Tubularia* showed little ChAc content despite its high ChE content.

The ratio of ChE to ChAc showed no constancy for the three species; rather the ratio progressively decreased as the nervous system became more highly organized. The problem as to whether this change is related to the functional state of the nervous system is worthy of further investigation.

Report of Investigations, Summer 1948. AVRAM GOLDSTEIN * AND DORA B. GOLDSTEIN.

I. *Non-nerve cholinesterases.* No reasonable function can as yet be attributed to the cholinesterases found in the plasma and liver of higher animals. This project sought to isolate and study the properties of a cholinesterase in a system where it would be known to have a definite metabolic function—e.g. in microorganisms. Fermenting cabbage, cucumber and algae (such materials had been shown to contain bacteria which *produce* acetylcholine—M. Stephenson, *J. Gen. Microbiol.*, 2, No. 1, 1948) were used as sources for the isolation by enrichment culture, of organisms which might utilize acetylcholine. Forty pure cultures were obtained (representing at least twelve different species) which grew in several passages through acetylcholine in mineral medium. These include Gram-negative bacilli, yeasts and molds. To date, growth and respiration studies reveal that some (but not all) metabolize added acetylcholine, grow on choline (but not on ammonia plus acetate) and hydrolyze acetylcholine anaerobically. One organism has yielded an acetylcholine-splitting enzyme in acetone powder. This esterase has not yet been characterized but it is insensitive to prostigmine. The work will be continued during the coming year.

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* Lalor Fellow.

II. *Modified Warburg technique.* Theoretical work was completed and experimentally confirmed for a variable-volume use of the standard Warburg apparatus. The method eliminates the necessity of repeatedly levelling the manometer fluid, increases the capacity for measuring gas uptake or liberation, and makes possible automatic recording of the progress of reactions.

III. *Further kinetic studies on the mechanism of inhibition of human plasma cholinesterase.* See Seminar, presented August 17, 1948.

The incorporation of P^{32} into the nucleoproteins and phosphoproteins of developing Arbacia embryos. C. A. VILLEE,* M. LOWENS, M. GORDON, E. LEONARD, AND A. RICH.

The quantitative conversion of ribonucleic acid (RNA) to desoxyribonucleic acid (DNA) in the developing sea urchin embryo was postulated by Brachet (1933) on the basis of an increase in DNA and decrease in RNA during the first 40 hours of development. Schmidt (1948) reinvestigated this with more precise analytical methods and found an increase in DNA but no decrease in RNA.

Sea urchin embryos obtained at stages from 3 to 72 hours of development and analyzed by the method of Schmidt and Thannhauser (1945) showed an increase in DNA with time whereas the RNA remained constant or increased slightly. The major source of the DNA formed cannot be the RNA of the unfertilized egg because when fertilized eggs are allowed to develop in sea water containing P^{32}, a great amount of radio-phosphorus is incorporated into DNA, which would not be the case if it were derived from the P^{32}-free RNA of the unfertilized egg. A comparison of the specific activities of the two fractions showed that for DNA to be formed from an RNA intermediate, less than 5 per cent of the RNA must be active in forming DNA and this small fraction must be synthesized and broken down completely more than four times in 24 hours.

A number of chemicals which have been found to have effects on phosphate metabolism were tested for their effects on phosphate uptake and on nucleoprotein synthesis by comparing the specific activity of the fraction of the treated with that of the control animals grown at the same time and under the same conditions. 5×10^{-5} M dinitrophenol and 10^{-2} M malononitrile inhibited cleavage so that after 12 hours only 1 or 2 cell stages were present, whereas the control embryos were free swimmers. Both 10^{-2} and 10^{-4} M malononitrile inhibited respiration to about 25 per cent of the control value. Uranyl nitrate and tris (β chloroethyl) amine hydrochloride inhibited cleavage but not, at the concentrations used, as much as dinitrophenol.

The table gives the effects of these substances on the incorporation of P^{32} into the several fractions after 12 hours of incubation. Dinitrophenol appears to inhibit the uptake of phosphorus by the cell (the acid-soluble phosphorus fraction is low) more than the synthesis of DNA and RNA. Low temperature, which inhibited cleavage completely, inhibited the uptake of phosphorus into the acid-soluble fraction about as much as dinitrophenol, but inhibited the synthesis of nucleoproteins much more. Malononitrile inhibited the uptake of phosphorus into the acid-soluble fraction to a lesser extent but inhibited DNA and RNA synthesis markedly. RNA synthesis appears to be more sensitive to malononitrile than DNA synthesis, which suggests that DNA is not formed from an RNA intermediate. Uranyl nitrate reduces the uptake of phosphorus and inhibits both DNA and RNA synthesis. The nitrogen mustard, tris (β chloroethyl) amine inhibited both DNA and RNA synthesis, DNA more than RNA, without reducing the uptake of phosphorus by the cell to any marked extent.

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* Lalor Fellow.

TABLE 1

Inhibition of the incorporation of P^{32} into the phosphate fractions of 12 hour Arbacia embryos

The figures in the table represent the values of the ratio:

$$\frac{\text{Specific activity (counts per minute per mg. P) of treated embryos}}{\text{Specific activity (counts per minute per mg. P) of control embryos}}$$

	Malononitrile		Dinitrophenol	Uranyl nitrate	Nitrogen mustard	Cold
	10^{-4} M	10^{-2} M	5×10^{-5} M	10^{-4} M	10^{-4} M	0° C.
Acid soluble P	.69	.49	.18	.49	.83	.16
Total acid insoluble P	.68	.10	.32	.40	.56	.06
DNA phosphorus	.55	.04	.10	.27	.23	.03
RNA phosphorus	.21	.03	.24	.40	.44	.03
Phosphoprotein P	.24	29	.16	.55	2.57	.23

Studies on nucleoproteins from marine invertebrates.[1] C. A. VILLEE,* E. LEONARD, AND A. RICH.

The studies on marine invertebrate nucleoproteins begun last summer were continued. Nucleoproteins were extracted from squid testis, spermatophore sac, and sperm receptacle using 2M NaCl. Purified solutions of these have a high relative viscosity and show strong flow birefringence. Measurements of the flow birefringence were made in the Mehl and Edsall apparatus. Changing the pH of these solutions to pH 3.6 or 8.5 did not decrease the viscosity or flow birefringence. Heating to 100° C. for 40 minutes did not decrease viscosity or birefringence but heating for 90 minutes at that temperature did destroy birefringence and decreased the viscosity markedly. There was no change in these properties on the addition of guanidine hydrochloride up to 20 mM/ml. solution, which suggests that these properties do not depend on the presence of free nucleic acid. Observations from the viscosity and flow birefringence data indicate that the nucleoproteins are present as markedly asymmetric particles. Using as a model an ellipsoid of revolution, calculations show a rotary diffusion constant of 1.5 and a particle length of about 10,000 Angstroms. It is possible that the nucleoprotein molecules are associated into larger particles under these conditions.

Analyses by the Schmidt and Thannhauser method for ribonucleic acid, desoxyribonucleic acid, and phosphoprotein were made on squid testis, spermatophore sac and on squid optic ganglion. The effect of continued stimulation on the nucleoprotein content of squid optic ganglia was investigated. Nitrogen : phosphorus ratios were determined on nucleoproteins and nucleic acids prepared from squid, starfish, and sea urchin tissues.

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* Lalor Fellow.

[1] Aided by a grant from the Ella Sachs Plotz Foundation.

PAPERS PRESENTED AT THE MEETING OF THE SOCIETY OF GENERAL PHYSIOLOGISTS

The wave of negativity produced by acetylcholine conducted over an oil-saline interface. T. C. Barnes and R. Beutner.[1]

The wave of negativity generated by acetylcholine at a phase-boundary has been found to travel from the point of application of the drug to the recording non-polarizable electrodes placed a meter or more distant. An oil-saline interface is essential for the transmission of this wave since it will not cross a saline bridge. The aqueous medium was 0.9 per cent sodium chloride. In some cases sodium lauryl sulfonate or sodium benzoate was used to increase the sensitivity of the oil surface to the alkaloid. The elongated interface consisted of a glass tube containing the two layers with openings at one end for the introduction of acetylcholine and at the other for the electrodes leading to the electroencephalograph. The addition of 0.1 cc. of saline containing 0.1 mg. acetylcholine at the "entrance" produced a negative wave of 3 millivolts lasting 0.15 sec. recorded at the "exit" 80 or more cm. away. The glass tube can be replaced by two contiguous wicks, one containing oil and the other saline. A single wick of oil or a single wick of saline will not transmit the acetylcholine wave. The mechanism of transmission is being investigated. Controls of distilled water or saline applied to this artificial nerve do not produce the wave. An electrogenic organic base (acetylcholine mecholyl or thiamine) is essential to establish the initial phase boundary potential. It will be noted that no "membrane" potential is necessary for initiation or transmission of the wave of negativity. The acetylcholine potential was originally set up as a permanent "base-line shift" potential (Beutner and Barnes, *Science,* **104**: 569, 1946), later developed into a wave (Barnes, *Federation Proceedings* **6**: 73, 1947), and the experiments reported here describe the transmission of the wave. It is possible that nerve impulses and brain-waves are produced by a similar mechanism.

Apyrase activity of invertebrate marine muscle. Arthur Cohen [2] (*introduced by H. B. Steinbach*) (*by invitation*). Read by Title.

A comparative study was made of the rates of hydrolysis of adenosine-triphosphate by unpurified homogenates of various marine invertebrate muscles. The results of these tests are shown in the table below in which the values are expressd as micrograms of phosphorus liberated from ATP by 1 mg. of fresh muscle in 5 and 15 minute incubation periods. No significant relationship between smooth and striated muscles, the nitrogen content of the muscles, or their activity, could be derived from the wide range of enzyme activity. In each assay of enzyme activity approximately 0.5 g. of freshly excised muscle was homogenized in 5 cc. ice cold distilled water and 0.1 cc. aliquot taken for analysis. The calcium salt of ATP was used with final concentration of 0.0015 M. The labile 7′ P of the ATP was at least double the concentration of the P split by the enzyme at all times during the experiment. The pH of the medium was equal to 7.4 employing a veronal acetate buffer. The phosphate determinations were made by the Fiske-Subbarow method. For total nitrogen determinations the modified micro-Kjeldahl method of Ma and Zuazaga was followed. Incubation temperature of the enzyme mixture was 22° C.

Muscle	μ P/mg./5 min.	μ P/mg./15 min.	μ Total N/mg.
Thyone (retractor)	0.1	0.34	18.0
Phascolosoma (retractor)	0.5	0.90	21.2
Limulus (tail)	0.62	1.50	28.2
Pagurus (abdominal)	2.8	4.20	29.6
Pecten (adductor)	2.8	4.73	22.6
Mya arenaria (mantle)	3.6	5.88	29.5
Loligo Pealii (fin)	4.7	9.00	25.3

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[1] Department of Pharmacology, Hahnemann Medical College.

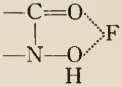
[2] University of Minnesota.

*On the nature of iron binding by siderophilin, conalbumin, hydroxylamine, aspergillic acid, and related hydroxamic acids.** Silvio Fiala and Dean Burk.[1]

Aspergillic acid, hydroxyaspergillic acid, N-hydroxy, 4-methylpyridene-2, N-hydroxy, 5-bromopyridene-2, N-hydroxy, 4,6-dimethylpyrimidene-2, and hydroxylamine have been found to bind ferric iron under appropriate conditions to yield complexes with the same absorption spectrum maximum (460–465 mμ) and order of extinction at this maximum (0.025–0.05 per cm. at microgram complex-Fe per ml.) as do the specific salmon-pink complexes of iron with conalbumin of egg white (Schade and Caroline, *Science,* **100**: 14–15, 1944) and of iron with siderophilin, the iron-binding β_1-pseudoglobulin of human plasma (Schade and Caroline, *Science,* **104**: 340–341, 1946; Helmberg and Laurell, *Acta Chem. Scand.,* 1: 940–950, 1947; Surgenor, Strong, and Koechlin, *J. Clin. Invest.,* in press; Schade, Reinhart, and Levy, *Arch. Biochem.,* in press).

Carbon dioxide (bicarbonate) is required to obtain the salmon-pink complex with iron and hydroxylamine, as Schade, Reinhart, and Levy first found for the iron complexes of conalbumin and siderophilin, the stoichiometric ratio of CO_2 to Fe in the latter being 1 : 1. With aspergillic acid and the related cyclic compounds listed, however, no carbon dioxide is required, consistent with the existence already of a carbonyl group adjacent to a hydroxylated nitrogen atom in the cyclic hydroxamic acid grouping that Dutcher (*J. Biol. Chem.,* **171**: 341–353, 1947) has indicated to be responsible for iron binding. The ratio of C=O to Fe in the salmon-pink aspergillic acid iron compound is 1 : 1.

Hydroxyl ion is also required to form the salmon-pink complexes of siderophilin and conalbumin, but it is not yet required by hydroxylamine and the cyclic hydroxamic acids listed, which already contain OH groups attached to nitrogen. The shape of the titration curve of the salmon-pink siderophilin complex, in the presence of buffer such as phosphate or citrate, indicates a ratio of OH^- to Fe of 1 : 1.

The grouping in the siderophilin, conalbumin and other complexes yielding an absorption spectrum maximum in the region 460 mμ might thus be represented as  $\begin{matrix} -\mathrm{C}{=}\mathrm{O}\cdots & \\ | & \mathrm{F} \\ -\mathrm{N}-\mathrm{O}\cdots & \\ \quad\ \mathrm{H} & \end{matrix}$. The specificity of the two proteins as compared with other proteins tends further to rule out primary involvement of the more common sulfhydryl and amino groups, which might otherwise be suspected of iron combination. Physiological implications will be discussed.

The cytochrome system in relation to diapause and development in the Cecropia silkworm.† Carroll M. Williams and Richard C. Sanborn.[2]

As soon as the pupa is formed, the metamorphosis of the Cecropia silkworm is interrupted by a prolonged period of diapause, which, at room temperature, continues for not less than five months. During this period the animal persists in a state of developmental standstill. The termination of dormancy is under overall control, being evoked by a combination of two internal factors. One of these arises from the pupal brain, the other from the prothoracic glands.

As a working hypothesis we may assume the existence of a biochemical defect in the tissues of the diapausing pupa that prevents their metabolism from contributing to morphogenesis. From this point of view, the brain and prothoracic glands should preside over some synthetic reaction whereby the tissues repair this defect.

Certain properties of the metabolism during dormancy and during development indicate that the biochemical defect of diapause may involve iron-catalyzed oxidations. Thus, during diapause, the oxygen consumption is essentially insensitive to cyanide. But when the brain and prothoracic glands function to terminate diapause, the oxygen consumption increases and a progressively larger fraction becomes cyanide sensitive. Consequently, development seems to involve a progressively larger utilization of the cytochrome system.

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* The siderophilin (plasma fraction IV-7) employed in this study was kindly supplied by Professor E. J. Cohn and Dr. Douglas Surgenor, Department of Physical Chemistry, Harvard Medical School, the conalbumin by Dr. H. L. Fevold, Western Regional Research Laboratory, and the aspergillic acid, hydroxyaspergillic acid, and related hydroxamic acids by Drs. J. D. Dutcher, O. Wintersteiner, and W. A. Lott of the Squibb Institute for Medical Research.

[1] National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

† This study was aided by the Lalor Foundation.

[2] The Biological Laboratories, Harvard University.

This possibility has been tested by spectrophotometric assay of cytochrome C and manometric assay of cytochrome oxidase. A striking correlation was found between the titer of these enzymes and the progress of adult development. Thus, cytochrome C is virtually absent from the diapausing pupa. During the period when development is dependent on the prothoracic glands, the concentration of cytochrome C increases from less than 1 to more than 50 gamma per gram live weight. Similarly, during the period of the brain's secretory activity, the titer of cytochrome oxidase increases from approximately 40 to nearly 700 units.

Two correlations seem to emerge; namely, that between the synthesis of cytochrome oxidase and the function of the brain, and that between the synthesis of cytochrome C and the function of the prothoracic glands. As a result of the combined functions of both the brain and prothoracic glands, the tissues of the dormant pupa, for the first time, come into possession of a complete cytochrome system.

The relation of heparin to protoplasmic clotting. L. V. HEILBRUNN AND W. L. WILSON.

An essential part of the present-day theory concerning the colloidal behavior of protoplasm is the fact that the gelation of protoplasm is in many ways similar to the clotting of blood. The changes which protoplasm undergoes during its normal activities and as a result of drug action are frequently to be explained in terms of a clotting reaction similar to that which occurs in blood. Additional evidence in support of this point of view is the fact that at least in some cells heparin can prevent protoplasmic gelation. In the egg of the worm Chaetopterus, there is a gelation which precedes the appearance of the mitotic spindle. If eggs are exposed to dilute solutions of heparin and then fertilized, the mitotic gelation is inhibited and for the most part the eggs do not divide. In order to show this, it is necessary to inseminate the eggs with high concentrations of sperm, for the presence of heparin tends to inhibit fertilization by weaker sperm concentrations. The effect of heparin on cell division is reversible. The fact that heparin can actually prevent mitosis is perhaps of some significance in the interpretation of the effect of radiation in the treatment of cancer, for following irradiation of animals, there is a marked increase in the amount of heparin in the blood stream. Moreover, heparin may be an important factor in the control of protoplasmic clotting in various other types of cell activity.

*Enzyme activity and radiation sensitivity of enzyme-substrate films.** DANIEL MAZIA AND GERTRUDE BLUMENTHAL.[1]

In a previous investigation (Mazia, Hayashi, and Yudowitch, Cold Spring Harbor Symp., 1947) the enzyme activity of mixed surface films of pepsin and albumin has been described. In all such experiments it has been difficult to exclude the possibility that the measured activity was not that of surface-spread enzyme molecules but of unspread molecules trapped in or adsorbed on the film. It has been found (Mazia and Blumenthal, *P. N. A. S.*, 34: 328, 1948) that these enzyme-substrate films are extraordinarily sensitive to radiation, and a study of the relation between activity, surface pressure, and radiation sensitivity throws light not only on the mechanism of radiation sensitivity but also on the question of the activity of surface-spread enzyme.

Methods are described in the publications cited. Surface pressure was measured by the vertical-pull balance. The activity of the films depends on the pressure against which they are spread. Films spread to zero pressure become inactive. Activity increases with initial pressure up to about 30 dynes/cm. There is some evidence of reversibility. The radiation-sensitivity is a function of the surface pressure. At a given dose, there is a discontinuous relationship between inactivation and pressure. Dose-inactivation curves at various pressures yield a family of sigmoid curves which show that the effect of pressure is primarily on the "threshold" of the dose-effect relationship. A plot of pressure against dose for 50 per cent inactivation yields a straight line. The 50 per cent dose at 7 dynes/cm. is about 100 r; at 35 dynes/cm. it is 500 r. The pressure effect on sensitivity is reversible. Sensitivity depends on the pressure at the time of radiation and is indpendent of previous pressure changes provided the pressure has not been permitted to fall to zero. Since not only the activity but also radiation-inactivation is a function of surface pressure it is concluded (1) that the spreading of proteins at an air-water interface ("surface denaturation") is not necessarily an all-or-none phenomenon, but may be limited by the pressure against which the protein spread and (2) that if the unfolding of the molecule is not permitted to go to completion (i.e. to zero pressure) the enzyme activity of the protein may be preserved.

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* Work supported by National Research Committee on Growth acting for the American Cancer Society.

[1] Department of Zoology, University of Missouri.

A photosynthetic intermediate. A. H. Brown, E. W. Fager and H. Gaffron.[1]

An intermediate of photosynthesis is by definition a substance which is transformed in one or more photochemical steps into the final product. This final product of photosynthesis must have the reduction level of a carbohydrate and be insensitive to further irradiation. The discovery of the dark fixation reactions in which free carbon dioxide becomes a carboxyl group in an organic molecule without the aid of light made it very probable that the first photosensitive intermediate in the course of photosynthesis is formed by a typical dark fixation and thus may be related to compounds which normally serve as intermediates of respiration. Consequently many biochemists expected photosynthesis to be revealed as a process in which intermediates known from the breakdown of carbohydrates are built up in steps which literally reverse the course of respiration.

Benson and Calvin have reported that in studying photosynthesis with radioactive carbon as a tracer they have obtained labeled dicarboxylic acids, amino acids and very considerable quantities of glyceric acid and of glyceraldehyde. According to these authors, these substances have been formed by the direct photosynthetic reduction of carbon dioxide, that is, neither by the breakdown of a previously synthesized carbohydrate nor by way of an ordinary dark fixation. The intimate relationship of respiration and photosynthesis as well as the pathway of the latter process thus seems to have been conveniently established.

In the course of our investigations with carbon 14 we could confirm most of the early observations of Ruben, Kamen and Hassid, but could not find any indication that the substances mentioned by Calvin et al. are present as intermediates of photosynthesis. Even in the case where these authors adopted the technique of short exposures and found glyceric acid and glyceraldehyde as main products, the discrepancy persists. Under the same conditions we obtain 90 per cent of the assimilated carbon in one chemical fraction of the plant. This fraction contains the carbon in a photosensitive substance (or group of nearly related substances) which is not identical with any one of the substances usually shown in the schemes representing the metabolism of carbohydrates. Labeled glyceraldehyde is absent, and glyceric acid is present, if at all, only in small amounts, which probably originate from secondary processes. The intermediate is obtained as a thermally and chemically very stable though impure syrup or its hygroscopic sodium or barium salts. It is easily adsorbed on all kinds of precipitates when these are produced in the solution. Its most interesting characteristic is its content of aromatic nitrogen. The ultraviolet spectrum is very similar to that of uracil, though this particular substance is not present. It seems that the radioactive carbon assimilated during the first moments of photosynthesis and a pyrimidine compound are closely associated. The chemical properties which set our photosynthetic substance apart from the better known simple metabolites are paralleled by its behavior in the living cell. Instead of being rapidly transformed in the dark like any respiratory intermediate it is stable against attack by respiration. It also does not readily exchange its labeled carbon with free carbon dioxide, as it should if it were a primary dark fixation product. But the labeled carbon begins immediately to appear in other chemical fractions when the plant is exposed to light. These results do not support the idea that the photochemical reduction of carbon dioxide proceeds by a simple reversion of each step in the breakdown of carbohydrates.

Synthesis reactions with acetic acid in isolated bone marrow. Richard Abrams, J. M. Goldinger, and E. S. G. Barron.

The metabolic activity of bone marrow has been the subject of much interest because of its role in blood cell production. It has been shown by Thorell (1947) that ribonucleic acid is associated with protein formation in the proliferating marrow; by Goldinger, Lipton, and Barron (1947) that marrow can oxidatively utilize acetate, and by Buchanan, Sonne, and Delluva (1947) that acetate carboxyl can be a purine precoursor (uric acid in pigeons). Accordingly, we have incubated rabbit bone marrow slices and homogenates with acetate containing C^{14} in the carboxyl group in an attempt to find an *in vitro* system for studying nucleic acid synthesis, as well as to survey the types of synthetic reactions in which acetate is involved. A small, but definite, incorporation of C^{14} was found in both ribonucleic and desoxyribonucleic acids. In addition there was observed a relatively large turnover in the phospholipid and protein fractions.

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[1] University of Chicago, Chicago, Illinois.

In a typical experiment, rabbit marrow slices were incubated for 5 hours at 37° in Ringer-bicarbonate containing $CH_3C^{14}OONa$. Taking 100 as the specific activity of the substrate, the relative specific activities of the subsequently isolated fractions were: ribonucleic acid (RNA), 0.035; desoxyribonucleic acid (DNA), 0.014; CO_2 liberated from hydrolyzed protein by ninhydrin, 0.61; lecithin, 0.90; and fatty acids from saponified fat, 0.10. A control experiment with $C^{14}O_2$ indicated that the startlingly high rate of protein formation from a fatty acid (as well as phospholipid synthesis) did not involve CO_2 as an intermediate.

The rate of incorporation of C^{14} was measured aerobically with slices and both aerobically and anaerobically with homogenates. With slices, there was a linear increase of specific activity with time in the DNA and amino acid carboxyl fractions, while the RNA activity, though reaching a level 3 to 4 times that of DNA, did so at a continuously diminishing rate. Homogenization caused a marked reduction in the rates of uptake of C^{14} by RNA and by protein. That respiration is necessary to furnish the energy for these synthetic processes is indicated by the fact that anaerobically C^{14} appeared in neither the nucleic acids nor the proteins.

Thermodynamic theory of the contraction of actomyosin. A. SZENT-GYÖRGYI.[1]

It has been shown in the speaker's laboratory that the contractile matter of muscle is built of a complex protein, actomyosin, composed of myosin and actin (F. B. Straub). Actomyosin contracts in a proper ionic milieu under influence of ATP. Evidence was obtained showing that the contractile matter is built of small functional units each of which consists of a certain amount of myosin, actin and one molecule of ATP. These units will be called "autones." Experiments indicated that these autones have but two stable states, the fully relaxed and fully contracted state; that contraction is an all-or-none process; and that it is an equilibrium-process, dependent on temperature. The equilibrium-constant was measured at different temperatures and free-energy changes calculated. It has been found that the ΔF, i.e. the free-energy spent by the single autone in contraction, rises with increasing temperature. In the rabbit it is 0 at about 0° C. and reaches 11,000 cal. at 53° C. In the frog-muscle, extracted with water, these values are reached a few degrees lower.

The first question is whether this ΔF curve is correct. The theory involves that the system uses its own F in contraction and extraneous energy is needed for relaxation. The extraneous source of energy is the high-energy phosphate of ATP which has 11,000 cals. of F. It follows that the muscle has to go over permanently into the contracted state at the temperature where the F-expenditure reaches or exceeds 11,000 cal., which is 53° in the rabbit and 47° in the frog. This was actually found to be the case.

In order to obtain further information about the correctness of the theory the total amount of work was measured in isometric and isotonic contraction. It was found that the curve of total work, if calculated for 35,000 gm. of myosin, agrees very closely with the ΔF curve. This is the case in isotonic contraction and in isometric contraction up to the point where the work corresponds to 5500 cal., ½ of 11,000. From this point on the tension developed remains constant and does not rise with increasing temperature.

The thermodynamic reversibility of contraction could clearly be demonstrated.

Having thus obtained evidence for the basic correctness of the theory an attempt was made to extend it. If we suppose that actomyosin, in absence of ATP, does not contract because the F of the contracted state is equal with that of the relaxed state, $F_c = F_r$, then contraction takes place with ATP because this latter makes $F_r < F_c$ which pushes the system towards a new equilibrium with a greater number of contracted units. Such assumptions can be tested by deriving their consequences and checking them in the experiment.

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[1] Marine Biological Laboratory, Woods Hole, Mass., and the National Institute of Health, Bethesda, Md.

$F_c = F_r$ involves an equilibrium constant of 1 which means that at the temperature in question there is one contracted unit for every relaxed one, even in rest. The contracted units, being folded, can be expected to be highly elastic, the relaxed ones to be inelastic. The relative length of units in the folded and relaxed state is 1:7. The limit of elastic extensibility is reached thus when the folded units are fully extended which is reached at 175 per cent of the equilibrium length. The experiment showed that this is actually the case.

The muscle is highly elastic only while it contains its normal amount of ATP. If this is decomposed the muscle becomes inelastic. That the change is actually due to ATP can be shown by adding ATP to the inelastic washed muscle fibers which, under influence of ATP, become highly elastic again. The elasticity of fresh muscle proves that ATP is present in an active form, linked to actomyosin.

If the fully contracted autone is stretched it offers in the beginning little resistance. Then the resistance becomes proportional to stretching (Hooke region) and in the end tension exceeds stretching. If the uncontracted units contract in an isometric contraction the contracted units are stretched. If the muscle would be at equilibrium-length it could in the beginning develop no tension. Rest-length means a stretch of these units which brings the muscle into the Hooke region. The maximum of tension is reached when all units are half-extended and tension is one-half the maximum theoretical value as has actually been found in the experiment. No higher tension could be developed by the muscle without its permanent damage. If this one-half tension is exceeded, slipping begins, and if the full tension is reached, the muscle tears.

The size of the autones can be approached in different ways. All observations indicate that it contains 18,000 gm. myosin, one molecule of ATP and 6000 gm. of actin. The actin and myosin in this unit are linked by one SH bridge. It is not impossible that these single autones also form higher units, containing one molecule actin of MW 70,000 gm. and thrice that amount of myosin.

Usnic acid, an antibiotic, and sperm metabolism. Leonard Nelson, *by invitation.* Read by Title.

The effect of Usnic acid on the respiration of Arbacia sperm was determined manometrically. This antibiotic had previously been shown to inhibit cleavage and P^{32} uptake in fertilized eggs of the same species, but had little effect upon their oxygen consumption (Marshak and Harting, J. C. C. P., 1948). However, the same concentration of Usnic acid pronouncedly affected the sperm respiration. This effect was found to be dependent upon the density of the sperm suspension. The sperm concentration was measured spectrophotometrically—the optical density being directly proportional to the logarithm of the number of sperm.

A concentration of 1 mg. of Usnic acid per 100 cc. of 0.01 per cent gelatin-sea water was employed throughout, while the sperm concentration was varied from 100,000 to 1,000,000 sperm per cubic millimeter. At 25° C., the respiration was completely inhibited in the low concentrations and in the intermediate and high concentrations there was a marked increase in oxygen uptake.

Sperm count / Sperm No./c.mm.	Per cent control		
	30 min.	90 min.	180 min.
100,000	0	0	0
180,000	100	0	0
250,000	470	375	280
410,000	565	590	660
1,120,000	470	500	500

Preliminary observations seem to show that these effects are not attributable to change in motility inasmuch as after a given interval, treated and control sperm had moved an equal distance in capillary tubes.

These experiments seem to indicate that some energetic process apparently independent of sperm motility and distinct from oxidative cycles in the egg is being affected. Similar results were obtained with Asterias sperm.

Temperature coefficients of Apyrase systems from muscles of different animals. H. BURR STEINBACH. READ BY TITLE.

The Apyrase activities of unfractionated homogenates of muscle were determined at various temperatures within the range zero to thirty degrees C. Reaction mixtures of 2 ml. volume contained 0.05 M barbital buffer, pH 7.4, ATP (as the Ca salt), 2×10^{-4} M as well as the enzyme preparation. Checks with some of the tissues with glycerophosphate as substrate showed negligible activity toward this simple ester. Ten minutes reaction time was used, the reaction being stopped by adding trichloracetic acid to a final concentration of 5 per cent.

Logarithms of rates at different temperatures plotted against either degrees C. (for Q_{10} calculations) or the reciprocal of the absolute temperature approximated straight lines with a few instances which might be interpreted as breaks at critical temperatures near 15° C. Average results for Q_{10} were as follows: for fish (*Clinostomus elongatus* and *Lepomis macrochiris*) 1.4 to 1.6, frog (*Rana pipiens*) 1.7, mouse 1.8 to 2.0, bird (*Passer domesticus*) 2.1 to 2.2, and turtle (*Chrysemys marginata*) 2.1 to 2.2. Further study would be necessary before concluding that these are real genetic differences between the animal groups. Preliminary experiments indicated that the Q_{10} values bore no particular relationship to the environmental temperatures of the animals since fish kept at 22° C. for one month gave preparations having the same Q_{10} as similar fish kept at 0 to 5° C. for a similar period. Likewise, tropical fish (*Xiphophorus hellerii* Heckel) had temperature coefficients nearly like those of cold water minnows.

A few observations of apyrase activity of brain homogenates of fish, mouse and frog showed uniformly low Q_{10} values of about 1.4.

Studies of the kinetics of potassium exchange between cells and plasma of canine blood in vitro using K^{42}. C. W. SHEPPARD AND W. R. MARTIN.[1]

Freshly drawn heparinized canine whole blood is equilibrated *in vitro* (paraffin lined vessels) at 38.1° C. with a normal pulmonary atmosphere saturated with water vapor. It is then tagged by mixing with a small amount of plasma containing dextrose and radioactive KCl. The potassium content of the resulting plasma is thus raised by not more than 3 per cent of the normal and the blood sugar to about 400 mg. per cent. By this procedure cells are maintained in a healthy state for periods up to 10 hours as shown by minimal hemolysis, nearly constant hematocrit and minimal potassium leakage.

In whole blood the activity of the plasma decreases initially at a rapid rate which is correlated with the appearance of activity in the cells (red cells, white cells, and platelets). The initial decrease follows an exponential curve to an elevated base line, half of this change being complete in about 50 minutes. However, if the white cells and platelets be previously removed by repeated differential centrifugation the behavior is different. The plasma activity declines very slowly, at the rate of about 1 per cent per hour.

The initial rapid decrease in plasma activity for whole blood is attributed to a rapid exchange of potassium in the fraction containing the white cells and platelets. A typical value for the amount of this easily exchangeable potassium is about 30 per cent of the total potassium in the plasma. It is evident that studies of potassium exchange of erythrocytes must give equivocal results unless the white cells and platelets are removed in advance or otherwise taken into account.

[1] Oak Ridge National Laboratory, Oak Ridge, Tennessee.

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THE LIFE CYCLE OF ASELOMARIS MICHAELI, A NEW GYMNOBLASTIC HYDROID

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The following account is of a hydroid not previously recorded, from the Atlantic coast of North America. It appears to be a form related to *Rhizorhagium* as redefined by Rees (1938), though not close enough to be included in that genus without a broadening of the definition and a reconsideration of the genera *Garveia* and *Bimeria.* Its closest relative appears to be *Atractylis arenosa* Alder (1862). According to Totton (1930), however, *Atractylis* is sunk in the synonymy of *Bougainvillia,* and *A. arenosa* is in any case in need of a new generic name. It is therefore proposed that the *Atractylis arenosà* of Alder become *Aselomaris arenosa,* and that the hydroid described here, which lacks the striking pseudohydrotheca and gelatinous perisarc of *A. arenosa,* be known as *Aselomaris michaeli.* This is in accordance with the views expressed by Rees and Totton (in personal correspondence). The genus *Aselomaris* is consequently defined as follows: bougainvillid hydroids with hydranths arisng singly from creeping stolons, with gonophores reduced to sporosacs and arising from the hydranth stalk, not from the stolons.

The present species was found throughout the general region of Boothbay Harbor, Maine, attached to the sides of floats, occasionally on Fucus fronds, in widely separated localities, namely, Lobster Cove, Townsend Gut, and the Town wharves. In every place it was associated with *Bougainvillia superciliaris* which it resembles in some respects, such as color, size and form of the hydranths.

Concerning its distribution, either it is an extremely local species or it has been extensively overlooked elsewhere. While a shallow water form, it is undoubtedly most inconspicuous. At the same time it should have been found if it occurred in the intensively collected Woods Hole region. Collections to the north have been more sporadic and its presence is therefore in doubt. It is quite possible that this is a northern species extending down to but not south of Cape Cod.

ASELOMARIS MICHAELI

This hydroid forms an encrusting mat often several centimetres square but with very little height. In fact most of the specimens obtained were collected only by shaving off the wood to which they were attached. The general nature of the hydranths is shown in Figure 1A. The hydranth grows vertically from the creeping stolon, the perisarc stopping short just above the base of the hydranth proper. It

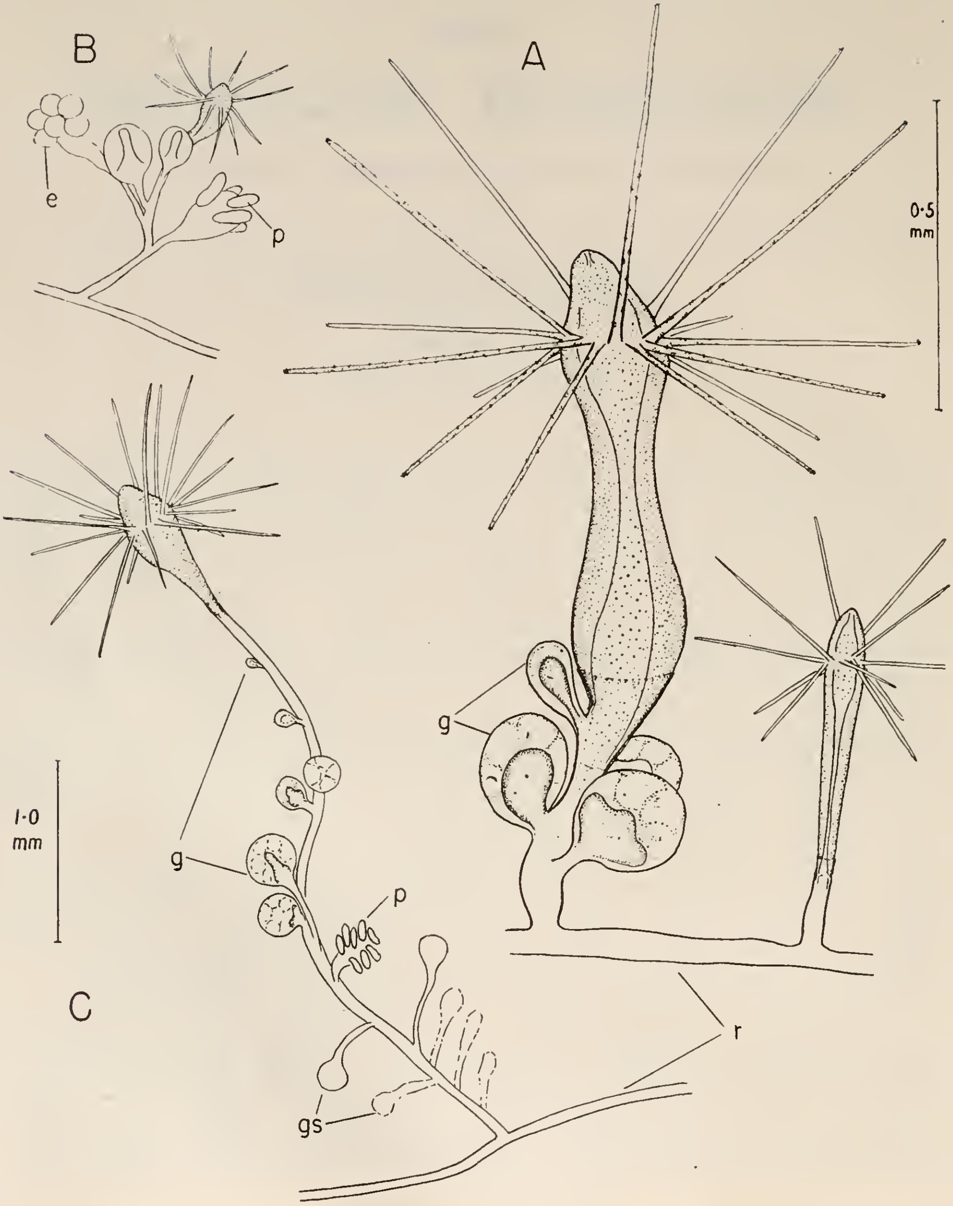

FIGURE 1. *Aselomaris michaeli.* A, large and small hydranths arising from the creeping hydrorhiza. B, somewhat older hydranth, at reduced scale, showing developing gonophores, gonophore with developing eggs and gonophore with planulae. C, maximum length hydranth, at same scale as B, showing succession of gonophores from distal juveniles to proximal stalk remnants. e, eggs; g, gonophore; gs, old gonophore stalk; p, planula; r, hydrorhiza.

has the form typical of the Bougainvillidae, with a conical manubrium and a single whorl of filiform tentacles. The tentacles are characteristically directed distally, laterally and proximally with virtually the same number in each category (Fig. 1A).

New hydranths grow only from the creeping stolon. Gonophore buds grow laterally from the base of the hydranth immediately proximal to the junction of naked and perisarc-covered coenosarc. This is a region of growth in a double sense. Not only are gonophores initiated, but the hydranth stalk grows in length, in effect pushing the growing gonophores down the stalk; that is, the new stalk that is progressively added proximally secretes chitinous perisarc and carries with it the growing gonophore, while new material and new gonophores are successively added between the first formed gonophore and the hydranth base. Thus we get a continually lengthening stalk bearing a series of gonophores, youngest nearest the base of the hydranth and oldest near the junction of the stalk and hydrorhiza (Fig. 1C).

No branches are given off and the hydrorhiza forms a closely applied creeping stolon. The only outgrowths are the buds arising from the hydrorhiza that develop directly into hydranths; that is, gonophores do not develop directly from the hydrorhiza, but only single hydranths; whereas hydranths only rarely form as lateral branches of the stalk, in place of a gonophore.

In an old hydranth as many as fifteen gonophores may be present, the youngest near the hydranth base being in early stages of devolpment, those in the middle zone being close to or actually functional, and those near the base of the stalk being represented mainly by stumps within a persisting perisarc.

FEMALE GONOPHORES

Gonophores first appear at the base of the hydranth at the region where the hypertrophied gastrodermis of the hydranth thins down to that characteristic of the coenosarc. They arise one at a time as a two-layered protrusion of the body wall. The entocodon, which is apparently of epidermal origin, arises relatively early. By the time the next in series is starting to form, a given gonophore has its entocodon differentiated almost entirely into about eight or ten oocytes. The more opaque endodermal component becomes somewhat pointed at the center, foreshadowing the spadix (Fig. 2B). With further growth the spadix forms a high cone, reaching to the distal epidermis of the gonophore (Fig. 2C). At its maximum size (Fig. 2D), the oocytes are full grown with the germinal vesicle of each lying in the part farthest away from the spadix. The gonophore stalk is greatly lengthened and is about twice as long as the diameter of the gonophore proper. Rhythmical movements or a writhing of the gonophore commences at this stage, at least under the conditions of microscopic examination. The contained eggs change shape with the movements and may appear themselves to be responsible for them, but a close examination reveals the activity to reside in the layer of the gonophore wall immediately beneath the epidermis and derived from the entocodon. In mature gonophores the writhing movements and contractions of the wall culminate in its rupture distally (Fig. 2E). This rupture has two consequences. Distally, where the cells become greatly stretched and thinned out, rupture results mainly in their disaggregation, so that instead of withdrawing as a sheet they remain as isolated spherical cells scattered over the sticky surface of the ripe eggs. The proximal part of the wall however does withdraw towards the base of the spadix, leaving the latter naked and still holding on to the eggs by adhesion. At the same time the epidermis, with its tension now released, contracts down the stalk, and where the stalk narrows the epidermis becomes somewhat thickened and wrinkled (Fig. 2F, G). The eggs are fertilized and undergo their cleavage and development up to the planula stage adhering to the gonophore spadix, remaining there until able to move away as a result of their own efforts. Planulae on the point of departure are shown in Figure 1B, C and Figure 4A. Throughout their development on the spadix, an internal active hydroplasmic streaming is maintained within the lumen of the spadix. Whether of course this streaming is of any value to the developing eggs is difficult to determine.

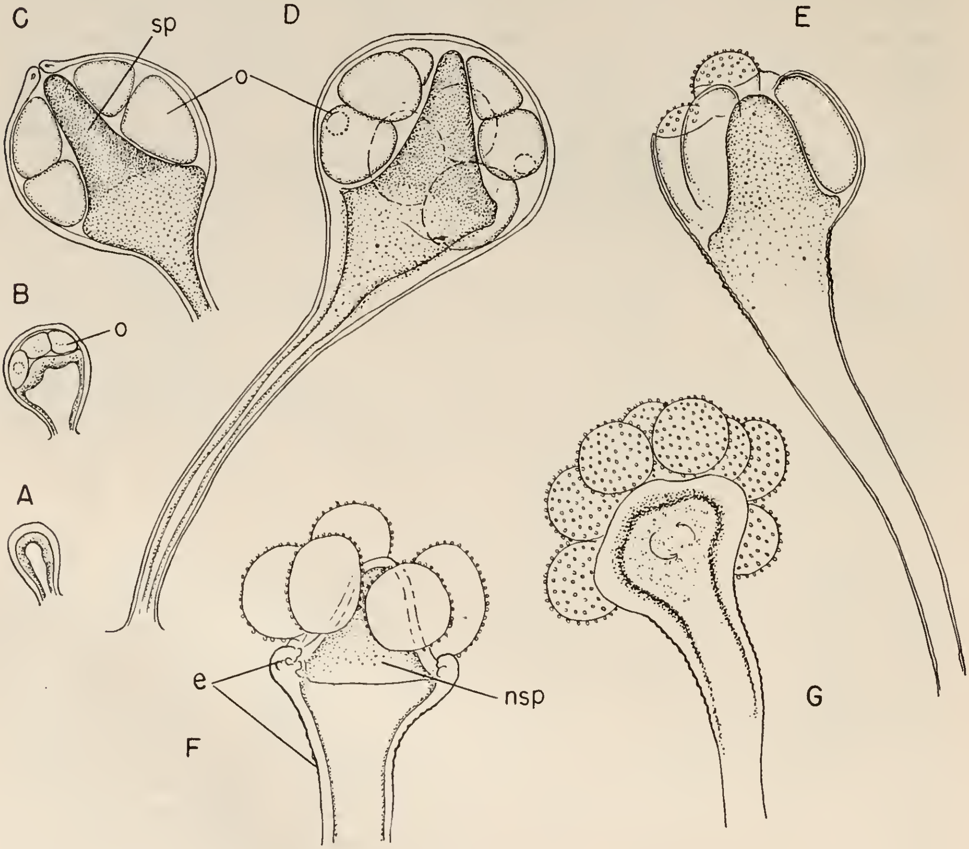

Figure 2. Female gonophores. A, young gonophore before appearance of entocodon. B, after segregation of ova from entocodon. C, D, fully formed gonophores with large ova and well developed spadix and long stalk. E, distal disintegration and rupture of epidermis, and basal epidermal contraction. F, G, matured gonophores with fully retracted epidermis, bearing fertilized eggs attached to the denuded spadix. e, contracted epidermis; nsp, naked spadix; o, ova; sp, spadix.

MALE GONOPHORES

The male gonophores develop as do the female, and have essentially the same appearance. Early stages showing the entocodon and the formation of the spadix are shown in Figure 3A and B. The lumen of the spadix alternately expands and contracts in a regular manner, with an amplitude indicated by the arrows in Figure 3C. In the later stages (Fig. 3D) the dilatation and contraction is more obvious in the short stalk of the gonophore, the growing mass of male germ cells possibly inhibiting or at least reducing the freedom of movement of the spadix wall itself.

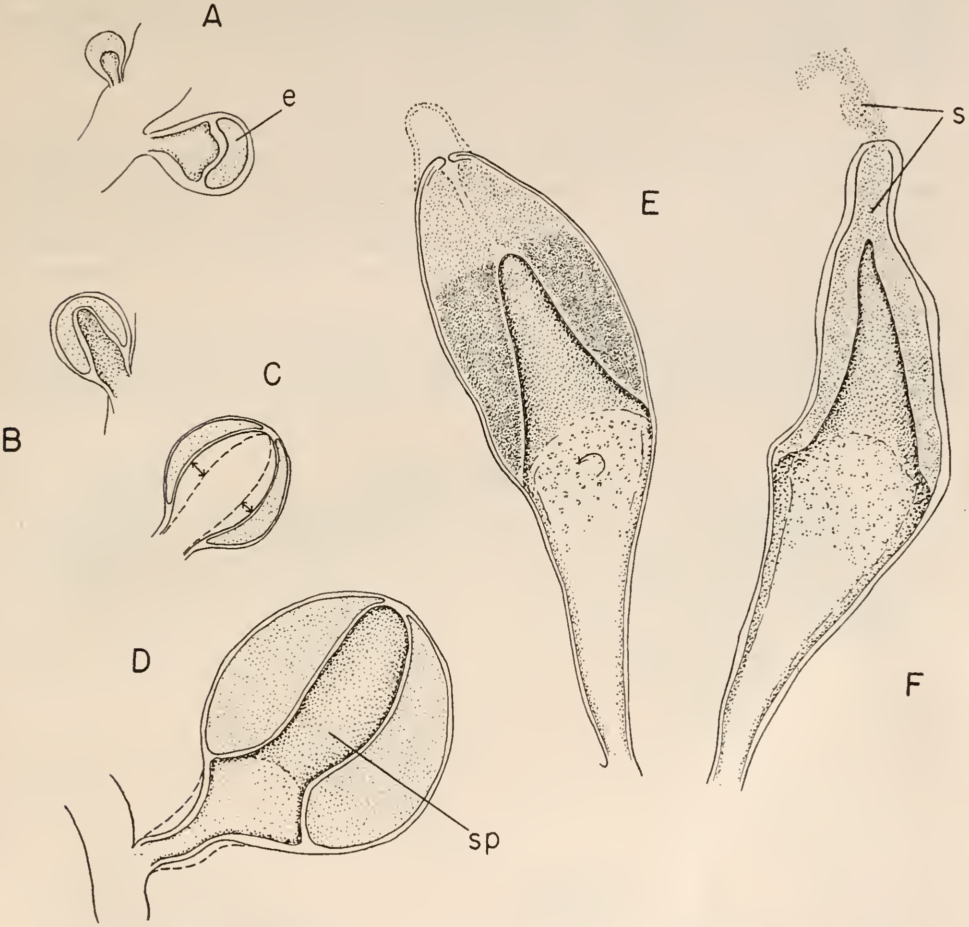

FIGURE 3. Male gonophores. A, B, young stage showing formation of entocodon and germ mass. C, later stage with arrows indicating expansion-contraction amplitude of spadix. D, late stage with germ cells present as spermatids, and broken lines indicating stalk diameter when dilated. E, ripe gonophore with active spermatozoa and expanding and contracting distal end. F, emission of spermatozoa. e, entocodon; sp, spadix.

With the attainment of full size, not only is the stalk relatively long, but the distal part of the gonophore also elongated. This is due on the one hand to the contractile property of the wall of the mature gonophore, and on the other to the pseudo-fluid quality of the mass of ripe germ cells. There is a rhythmical contraction of the gonophore wall similar to that of the female gonophore, but here resulting in an alternation between the stages indicated in Figure 3E. Finally the contractions culminate in rupture at the extreme distal pointed end, and the consequent escape of mature spermatozoa, shown escaping in Figure 3F.

Liberation of Eggs and Sperm

Both mature eggs and spermatozoa are liberated in essentially the same way, even though the eggs are not actually set free in the process. Rhythmical contractions of the gonophore wall result in its rupture distally. The contractions in each case are due to the activity of the tissue immediately subjacent to the epidermis, which must be regarded as homologous with the muscle layer of the free-swimming medusae of species of Bougainvillia.

Spawning in hydroids is usually associated with dawn (Lowe, 1926) or dusk, and not with darkness. Yet most of the ripe gonophores were examined at night and most of them ruptured within five minutes of first being exposed to the micro-

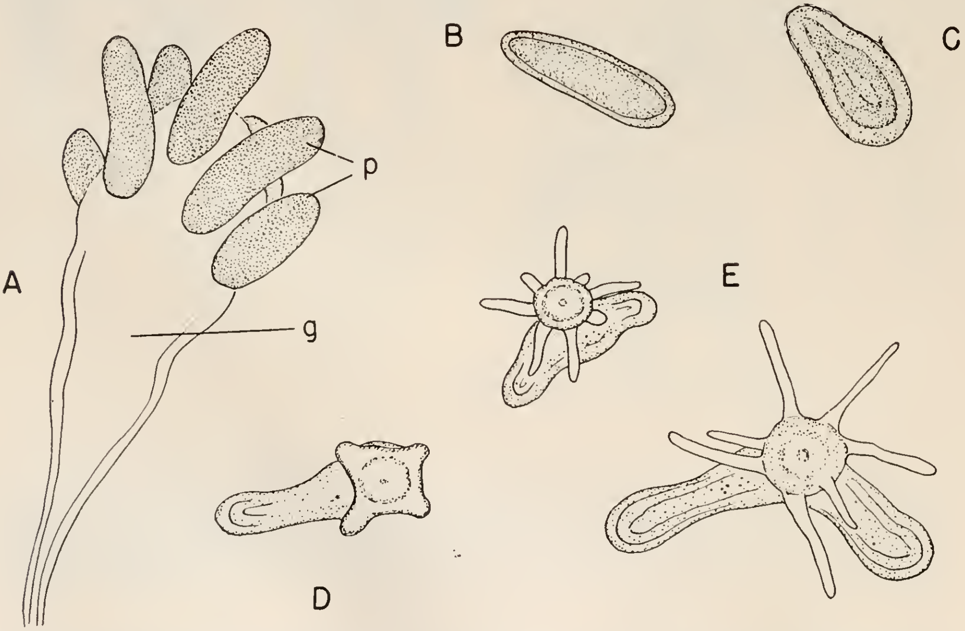

Figure 4. Development of planula. A, planulae about to swim away from gonophore. B, free-swimming planula. C, planula after about 24 hours, changing shape and losing ciliation. D, 12 hours after attachment, with stolon and 4-tentacled hydranth. E, two individuals 24 hours after attachment, with bipolar stolon on hydrorhiza and 8-tentacled hydranth. g, gonophore; p, planula.

scope light. It appears probable therefore that the stimulus of light evokes the contractions of the muscle layer, and spawning inevitably follows.

Settling of the Planula

Planulae escape from their adhesion to the spadix only as they become ciliated and active. A set about to launch forth is shown in Figure 4A. A planula swims for about 24 hours as a ciliate organism, and then becomes progressively pear-shaped (Fig. 4C), at the same time resorbing the external coat of cilia. About 12 hours after settling, a hydranth and stolon are already differentiated (Fig. 4D), four tentacles emerging in the first place. Twenty-four hours after settling, four intermediate tentacles are usually well formed, or a total of eight, while stolonic growth is bipolar, growing in opposite directions from the base of the hydranth along the substratum (Fig. 4E).

Summary

A new species of a hydroid genus not previously recorded from the Atlantic coast, *Aselomaris michaeli,* is described. The development and activity of both male and female gonophores are described in detail, together with settling of the planula and formation of the first hydranth.

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NOTE ON THE SPAWNING OF THE HOLOTHURIAN, THYONE BRIAREUS (LESUEUR)

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Thyone briareus is fairly abundant at Woods Hole, Massachusetts, yet very little has been recorded about its spawning. The present note is based upon observations made in the laboratory in 1948.

The Breeding Season

June is the principal month in which shedding has been observed in the laboratory at Woods Hole. Pearse (1909) noted spawning from June 22nd to July 5th. Ohshima (1925) reported it from June 21st to 24th in 1921, and Just (1929) observed it repeatedly during the month of June for several years. Mead (1898) found every animal full of nearly ripe eggs or sperm on April 24th, which would suggest a season beginning earlier than June, but Just (1929) claimed that eggs obtained in April and May were unripe ovocytes, capable of responding to insemination but unable to develop. There are no other data for the early part of the breeding season. As to the latter part, Pearse's observation of shedding on July 5th is the latest specific data published for *Thyone* at Woods Hole, although Bumpus (1898) remarked that the breeding season was probably June and July and Clark (1902) stated that *Thyone* apparently bred in the summer.

A study of 314 animals was made during the second half of June, 1948. Forty-nine of these animals shed in the laboratory, and 215 which did not shed were dissected. The results are summarized in Table I. The table shows the number of animals collected on each date and their condition as determined by shedding or dissection, together with estimates of shedding capacity based on the findings in these two categories. Line 8 in the horizontal direction includes some cases of undetermined sex among spent animals. When spawning is over, the gonadal tubules are so small that a careful microscopical examination is necessary to determine the sex. Such examinations were not made in the cases indicated. The single case of undetermined sex among the partly spent animals of the June 19th group was simply the result of an oversight. It is quite easy to distinguish the sexes when partly spent animals are dissected. The gonadal tubules of the male are an opaque yellowish or orange color and are pointed at the distal end, while those of the female are more translucent, golden or mustard colored, and have blunt distal ends. The dates of collection shown in the table for the different groups of animals do not necessarily indicate the dates on which spawning occurred. In fact, owing partly to experimental conditions, sheddings usually took place on subsequent dates. Nevertheless, the sheddings are listed as of the date of collection inasmuch as they indicate, no matter when they occurred, the shedding *capacity* of animals collected at that time. For example, some animals collected on June 15th were

TABLE I *

Shedding data and estimates of shedding capacity. (Explanation of categories in text.) Since shedding occurred under various experimental conditions the numbers in line 5 do not necessarily reflect maximum shedding as it might have occurred in nature.

1. Group	1			2			3			4			5			6			8			10		
2. Date	June 15			June 19			June 21 or earlier			June 21 or earlier			June 23			June 26			June 29			July 15		
3. Number in group	24			64			35			48			36			63			20			24		
4. Sex	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	♂
5. Number shed	1		8	3		3	3		15	5		5	0		0	2		3	0		1	0		0
Number dissected																								
6. Full	0		0	5		0	0		0	2		0	1		0	0		0	1		0	0		0
7. Partly spent	4		5	7	1	14	1		2	0		7	0		4	7		13	0		4	0		3
8. Spent	1		0	11	4	15	2		1	7		6	15		9	21		11	6		4	10	6	5
9. Remainder estimated sheddable	1.3		3.4	.23		.26	1.8		7.8	3.8		6.0	.24		.96	.93		1.66	.25		1.25	0		0
10. Estimated spent	.3			.5			1.4			6.2			5.8			3.4			2.5			0		
11. Number of each sex estimated in group	10.9		13.1	29.1		34.9	15.9		19.1	21.8		26.2	16.3		19.6	28.6		34.3	9.1		10.9	10.9		13.1
12. Number of each sex estimated sheddable	6.3		16.4	15.2		17.3	5.8		24.8	10.8		15.5	1.3		4.96	9.9		17.7	1.3		6.3	0		3
13. Per cent of each sex estimated sheddable	57.9		125.5	52.4		49.5	36.7		129.9	49.4		59.3	7.6		25.3	34.6		51.5	13.8		57.3	0		22.9

* Note. Total number of females (shed and dissected) = 115, or 45.4 per cent females
Total number of males (shed and dissected) = 138, or 54.5 per cent males
63.5 per cent of the females studied were spent
36.9 per cent of the males studied were spent
7.8 per cent of the females studied were full of eggs
None of the males studied were full of sperm

found shedding on the 18th, others not until the 22nd, 23rd and even the 26th of June, yet all of these sheddings indicate the shedding capacity of animals collected on the 15th. Compared with specimens actually collected on the 23rd or 26th, the June 15th group shows a much higher shedding capacity. The numbers collected were meager for some of the dates studied but an analysis of the table does show, in a general way at least, the probability of obtaining embryological material at this season. It may be mentioned that no single season is necessarily typical, since there is considerable variation in weather conditions during the spring months from year to year and this is probably reflected in the environment of the *Thyone* beds. A comparison of water temperatures at Woods Hole for the years 1902 through 1906 is given by Sumner, Osburn, and Cole (1911) and the air and water temperatures of recent years are on file at the Marine Biological Laboratory and the Woods Hole Oceanographic Institute (unpublished).

It has not been found possible to determine the sex of *Thyone briareus* by external inspection. Certainly there are no obvious correlations between sex and size, color, general appearance, or behavior. Therefore it is assumed that the specimens selected for dissection represented a random sampling of the entire group and showed about the same proportions of the two sexes as would any other sampling, as, for instance, the specimens left undissected. From lines 5, 6, 7 and 8 it can be seen that a total of 115 females were found, through shedding and dissection, while there were 138 males. Obviously 253 animals are not enough for a very accurate study of the sex ratio but, for want of more, this number must be used at present. Hence, it is concluded that 45.4 per cent of the animals would be females and 54.5 per cent would be males, or a ratio of about five to six, in any group of *Thyone* collected as were the subjects of this study.

Estimates of shedding capacity within a group were obtained in the following manner. (1) Animals which actually did shed were considered sheddable as of the date collected. (2) Some animals were probably prevented from shedding by the conditions to which they were subjected. If dissection showed the gonads full of seemingly mature gametes, the animals were considered sheddable. This seemed permissible since shedding did occur in the first group collected. (3) It was found subsequently that a given animal can shed more than once, at intervals of several days. Therefore, dissected animals found to be only partly spent were also considered sheddable. (4) Some animals that did not shed were not dissected, but an estimate was made showing those that should have been able to shed. (The number not examined was multiplied by the proportion found sheddable among those that were examined.) The sum of these four categories gave the estimated number of animals able to shed, from which the percentage of shedding capacity for the group could be calculated. However, since there were more males than females and since, moreover, most groups showed more females than males to be already spent, the shedding capacity was estimated in terms of percent of each sex capable of shedding. This seemed worth while in spite of the inaccuracies bound to arise from a study of such small numbers (e.g. Group 3 shows 129.9 per cent sheddable males), because the low female shedding capacity would otherwise have been masked by the higher male activity.

Table I shows that nearly half of the females examined in the middle of June had already shed their eggs. By June 21st more than half were spent, and as early as June 23rd one group showed the exceptionally low shedding capacity of only 7.6 per cent. In the last week of June, little more than a third of the females could shed and much lower percentages might be expected, such as the 13.8 per cent noted on June 29th. By July 15th there were no females able to shed, out of a group of 24 animals examined, although three of the males could still have shed some sperm. Aside from the numerical evidence of a waning season, the quantities of mature germ cells, either shed or found by dissection, dwindled as the month progressed. It seems, then, that the guess of Bumpus about shedding in June and July in 1898 would only have proved half accurate in 1948. And while one might have expected an occasional shedding of eggs as late as July 5th, as Pearse reported in 1909, that date could not have been recommended for the purpose this year. In fact, it would not have been worth while to attempt a detailed examination of the eggs of *Thyone* from animals collected after the third week of June.

Although this study was not begun until the middle of June, there are some indications of what may have occurred earlier. One might expect a higher percentage of mature females to be capable of shedding at the height of the season than actually did shed at any time from mid-June on. Nearly half of the batch was spent as early as June 19th. As early as June 15th there were as many partially spent as there were shedding, and on the 19th and again on the 23rd, there were more partially spent than the sum of the shedding and fully ripe ones combined. Certainly the season was waning during the entire second half of June. All this suggests a peak reached before June 15th. If this is correct, then the spawning season must either have been very short or have begun before June 1st. We have Mead's (1898) observation to support the latter possibility and Just's (1929) to support the former. Until the question is settled, the most promising time for successful embryological collecting must be considered to be the first half of June or possibly the middle two weeks of this month.

Shedding

Before it begins to shed, an animal expands greatly, to perhaps twice its former length, and starts waving its tentacles gently in the water in "feeding movements," as noted by Pearse in 1909. The position of the body can vary from vertical to horizontal, attached to the bottom or the sides of its container. It needs enough free space for the extended tentacles because sudden contact with any object will usually cause contraction, which might possibly delay shedding. On the other hand, shedding is not impossible in cramped quarters and once begun, under whatever circumstances, the process can be resumed in spite of various interruptions, such as removing the animal from water, leaving it practically dry, placing it under a very bright light, rinsing it in cold tap water, and so on. However, the writhing movements of an animal out of water or in too small a container will disperse the germ cells and could injure them.

As has been mentioned above, the sexes are indistinguishable externally. In both, the genital pore lies at the tip of an inconspicuous little stalk in the mid-dorsal line, between the bases of two large tentacles. It is easily located since it occupies the point on the tentacular ring diametrically opposite the only pair of small tentacles. The genital duct connects the pore with the gonads, which lie along the dorsal body wall, about halfway between the mouth and anus. Kille (1939) has described the gonads in post-spawning animals and discusses the tubules and their contents as found in July and August. It seems as if the entire gonadal knot need not be involved with spawning each year and some tubules may remain small and undistended. On the other hand this may be simply the appearance of late-season tubules. Ohshima (1925) described irregular masses of disintegrating yolk in the ovarian tubes, concluding that they were probably eggs which had failed to be laid in the previous spawning season and were undergoing degeneration. Brownish masses were especially notable in the ovarian tubules of spent females toward the end of the present study and these too were given Ohshima's interpretation, but it was thought that they probably represented degenerating eggs of the current season. Kille suggested that some large tubules may have been lost by some of his animals seen in July and August, an impression also gained from the present study, but not actually proved.

Once when an animal was vastly expanded and undergoing the writhing movements that precede shedding, the body wall became semi-transparent and the genital duct could be seen, a straight, grayish line leading to the pore. It showed clearly even when the animal was removed from the water and held up to the light. Shedding finally did occur and the animal proved to be a female as had been predicted. After the shedding the line was gone, but later a shorter line was noticed in the same region. Since the eggs are grayish and the sperm white, it is possible that some method of sex diagnosis might be worked out, but it probably would be practical only with full, mature animals. It was not effective this season.

The germ cells are emitted in a slow, fountain-like stream, so that they rise upward for a very short distance and then fall toward the bottom of the container. Since the tentacles are waving, there is fairly rapid dispersal, especially of the sperm. Moreover, animals nearby in the same container will ingest the gametes being shed by a neighbor. A female which has been in a container with a shedding male can be partially freed of sperm by rinsing in running tap water; but since the tentacles are withdrawn during this process, all the sperm cannot be reached and some of her eggs, if shed soon, are certain to be fertilized. Many changes of sea water with intervals for feeding movements in each would cut down the possibility of sperm contamination, but not eliminate it. Probably the best way to obtain unfertilized eggs under these circumstances is to hold a slender medicine dropper directly above the genital papilla. If introduced slowly and gently this is quite feasible, although tedious. It is much easier to keep prospective shedders in separate containers. Segregation is desirable even if fertilized eggs are to be collected, since the relatively large amount of sperm, even in 6 by 9 inch battery jars, will literally smother the eggs and is to be avoided.

The duration of the shedding process varied considerably, perhaps depending upon the age, and hence size, of an animal as well as on its degree of fullness. When watched, the process was sometimes as short as 10 or 15 minutes but extended up to four and a half hours in the largest shedding witnessed. Usually it was completed within about a half hour of the time of starting. Sometimes shedding is not quite continuous even when apparently undisturbed, and will proceed intermittently with intervals of from a few minutes on up to nearly three days. These latter repeated sheddings were noted several times, in one unusual case 7 and 9 days, respectively, after the animal was collected. Repeated shedding has not yet been found in a female.

The present study confirms the statements of Ohshima (1925) and Just (1929) that eggs obtained by means other than natural shedding connot be fertilized in the laboratory. It is easy to secure large eggs simply by mincing the ovarian tubules. The eggs which fall out are about as large as shed eggs. They contain a large germinal vesicle which has never been seen to break down except under pressure, applied externally. They have not been fertilized.

Factors in Shedding

No reliable method has been found to induce shedding at the will of the investigator, but various factors which might influence the phenomenon have been examined in connection with the shedding of the 35 males and 14 females which took place during the present study. The preponderance of males shedding was much greater than might be expected on the basis of the sex ratio. Perhaps they simply shed more easily or more frequently. Perhaps their activity lasts longer during the declining season. Whatever the reason, it should be borne in mind that the factors considered were effective on males particularly. They cannot be clearly demonstrated to be shedding agents until they have been examined again, at a time when many ripe females are available.

(1) *Time of Day*

Ohshima (1925) found that shedding always occurred late in the afternoon of the day the animals were brought into the laboratory. Once in 1948 a number of animals did shed around 6:00 P.M. of the day they were delivered, but it happened that this batch had been collected previously and kept on the water table in the supply department for at least a day. By far the majority of sheddings watched this year took place in the evening, mostly around 8:00 and 9:00 o'clock, and some as late as 1:00 or 2:00 A.M. Of course, many of these sheddings occurred under experimental conditions and could not be attributed entirely to a natural tendency. It did seem, however, as if there might have been a rather strong natural bent toward evening shedding which persisted despite external conditions. Nevertheless, at least two animals were seen shedding in the morning, one between 9:00 and 10:00 o'clock and the other at 12:00. Therefore, this part of the day need not be ruled out as a possible time for obtaining the germ cells.

(2) *Light*

Ohshima was able to induce animals to shed during the day by placing them in a dim light. Returned to bright light, they ceased shedding but would continue again if replaced in subdued light. Utter darkness did not cause shedding. Presumably these observations were made during the four days when Ohshima obtained eggs. During the present study sheddings were observed on at least ten different days and under many circumstances. They were seen to take place in natural, indirect daylight, in subdued daylight and in electric light of various intensities from rather subdued to bright lamplight directed right on the animals. They were also found to have occurred in complete darkness. Several times a male which had started to shed was picked up unceremoniously and transferred to a new dish under a bright electric lamp. Soon it started to shed again. Some females shed in bright light and their performance was watched in light sufficiently bright to allow one to see it easily, but it is true that at least 8 of the 14 females studied did shed in either complete or semi-darkness. The number is too small to be significant. This diversity of findings seems to rule out subdued light as a prerequisite. Possibly Ohshima's observations can be explained in the following way.

Thyone is very sensitive to changes in light which occur suddenly, as when, as Pearse showed (1908), a shadow passes abruptly between the animal and the light source. It also reacts immediately to jarring. The reaction to both stimuli is the same: contraction and in-drawing of tentacles, enough to interrupt shedding. It may take a minute or longer before the animal will expand again after such a contraction. If the shedding time were a short one, interruptions such as these would be enough to appear to stop the process. With only four days of observations, and eggs to collect at the same time, Ohshima may have returned his specimens to the dim light before they had had time to start shedding in the other types of light investigated. On the dates of his collections, June 21st to 24th, he might also have been dealing with some animals that were partly spent and had little to shed, as in the present study.

(3) *Presence of a Male*

Finally, Ohshima found no females spawning spontaneously, but only after the emission of sperm by nearby males in the same container. This year it was inconvenient to isolate all the animals of a group because of the container requirements for animals of this size, but at least two females did start to shed while wholly isolated in separate bowls. Others, isolated as soon as shedding was noticed, would continue after isolation, and this was, incidentally, usually after a rinsing in cold tap water too. The male effect was tested conversely when a female which had shed two days earlier was placed in a jar of freshly shed sperm, in an attempt to induce further shedding. It did not occur, even though subsequent dissection showed at least some mature eggs in at least some ovarian tubes. It would be more conclusive to use a full, mature female, but as yet no way has been found to distinguish such animals. At all events, isolated females are perfectly capable of initiating shedding.

(4) *Size*

The size of these animals is very hard to determine since so much depends upon their water content at the time of examination. However, any animals known to the supply department as medium or large will probably prove satisfactory as a source of germ cells, in season. It is not always the largest in a batch that will shed first, nor is the size any criterion of the sex of an animal. Very small specimens are to be eschewed for several reasons. (a) If mature, they will have only a small quantity of germ cells. (b) They may be mature animals already spent and hence reduced in bulk by the absence of distended gonads. (c) They may be small because they have eviscerated and hence are lacking in most of their viscera, usually including the larger gonad tubules. All three types have been encountered in the present study, (a) and (b) most frequently.

(5) *Quantity of Sea Water*

Just (1929) observed spawning repeatedly among animals if kept in large quantities of sea water. This year, shedding would begin in any amount of sea water sufficient to hold the animal and allow it to expand, and once begun, several males were found able to continue shedding even when placed in practically dry finger bowls in the hope of stopping them temporarily. They provided a little water for themselves, from the cloaca. Sometimes an animal barely submerged in a four-inch, shallow bowl would undergo the expansion that usually precedes shedding and would virtually double up on itself in the cramped space. When noted, this was remedied out of sympathy and the animals went on to shed in larger quarters, but sometimes they were not moved, and shed in the small containers. Usually, however, shedding was observed among animals in the 6 by 9 inch or 6 by 7½ inch cylindrical battery jars which proved to be the most suitable containers for them. Here there was room for maximum expansion but at the same time some hope of retrieving the shed cells.

The water was usually changed several times a day to ensure freshness but it is questionable whether this is really necessary if the animals are stored in a cool place (15°–17° C.). One sturdy specimen was found shedding after at least two days in unchanged sea water at about 20° C. Pearse (1908) and others have shown that *Thyone* can endure a good deal of variation in the environment, such as higher salt concentration, dilution with fresh water, excessive heat (some survived more than two hours' exposure to as high as 37° C.), or exposure to air. It is unlikely, then, that large quantities of sea water are essential to shedding. However, it is probably desirable for storage, since animals stored in running sea water did shed, even many days after collection. It would be very hard to gather eggs or sperm shed at random in a large aquarium and, as a matter of fact, no sheddings were observed among stored animals. Possibly they escaped notice because of rapid dissipation of the gametes.

(6) *Freshness of Animals*

It is generally held that fresh, well-fed animals provide the best embryological material. In the case of *Thyone* there is one drawback to using animals just collected. Their habit of pumping mud through the gut soon clouds the water so that gametes would be very hard to see, if present. The waving of the tentacles provides an excellent stirring system which keeps the debris from settling. An animal which has been stored in a large aquarium, or in running sea water, overnight or longer, has got rid of much of this material and is an easier one in which to watch shedding. Spawning often does occur several days after collection and in a few cases animals which had been in the laboratory as long as nine and ten days were found shedding. Normal larvae developed from eggs shed two or three days after the parents were collected. The problem, then, is to keep the animals from shedding until after they have emptied their digestive tracts.

(7) *Temperature*

Warming. There is some evidence that temperature plays an important role in the spawning of *Thyone.* Several times when half of a group of animals was kept at room temperature (20°–22° C.) while the other half remained at the temperature of the sea water (16.5°–18° C.), a number of sheddings occurred among the warmed animals, none among those kept cool. Precise tests of this were not feasible this year because of the increasing number of spent animals among those collected. The most suggestive results were obtained with 34 specimens collected on or before June 21st. At 3:35 P.M. on that date, 17 animals, group A, were placed in water at room temperature while 17 others, group B, were left in the running sea water. Five and a half hours later the water was changed on group A, some of the animals being put into fresh cold sea water, the rest into fresh warm sea water. Within a half hour shedding began in the group returned to cool water, and an hour and a half later it began in the group continued warm. In all, there were 12 out of a possible 17 sheddings. Meanwhile the animals of group B, kept cool while the others were being warmed, were placed in containers of fresh sea water and under the same lighting conditions as group A (typical overhead electric light of this laboratory). Part were put into cool water and the others into water at 20° C. There was no shedding at all during the next three hours or indeed overnight after the animals had been returned to the cold water table. Yet subsequent examinations showed that at least some of these animals could have shed, though some were already spent. The warming, if this experiment is indicative, should be of longer duration than three hours and more in the vicinity of five and a half, or more, hours.

Further support for the belief that warming induces shedding comes from the totals of the whole season's studies: No sheddings were ever observed in animals which had not been warmed for some time beforehand. Unfortunately, however, many animals which were warmed failed to shed, and others did not shed the first time they were warmed, but only after warmings on several days or after staying warm continuously for several days. Perhaps results such as these may be attributed to the waning season, or to the possibility that animals kept in confinement for many days, especially after being warmed and cooled spasmodically, may react with less predictability than fresher ones. Additional exploration of this aspect of shedding is certainly needed.

Cooling after warming. A change to cool water following a period of warming often seemed to stimulate shedding, but since most of these cases occurred at the commonest shedding time, from 8:00 to 11:00 P.M., time of day could not be ruled out as part of the stimulus. The best favoring evidence was this: A group of animals which had been in the supply house for at least a day was brought to the laboratory at 1:20 P.M. on June 22nd. They were in water of 17.8° C. which rose to 18.8° C. by 2:50 P.M. At that time all the jars of animals were placed in a cooler in which the temperature was about 19° C. but was gradually descending. At 5:45 P.M. the temperature had reached 16.5° C. and a number of animals of both sexes were shedding. Warming followed by cooling had certainly occurred but the exact time of the two periods is not known. There were also other factors. The cooler was entirely dark while housing the animals and it was vibrating vigorously. The matter of light has been discussed above and may be dismissed here. In view of the animals' normal reaction to jarring (Pearse, 1908) it seems as if the shedding may have occurred in spite of this obstacle. The time at which the shedding took place is especially interesting. It was much earlier than usual. Could it be that the treatment had overcome another possible obstacle, namely, time of day?

Continued cooling. Just as warming, or warming followed by cooling, may possibly stimulate shedding, so continued cooling appears to prevent it from taking place. If freshly collected animals are not wanted for immediate use they should be kept as cool as the water they came from, that is, no warmer than 15°–17° C., to discourage shedding. On the other hand, once an animal has started to shed, sudden cooling does not seem to stop it.

(8) *Other Factors*

A few attempts were made to stimulate shedding by other, more artificial, means. External mechanical stimuli such as jarring, squeezing or pricking merely resulted in quick contraction with tentacle withdrawal, wholly unfavorable to shedding. More drastic treatment, like cutting, either caused contraction as above or evisceration which makes shedding quite impossible. Injections into the body cavity can be effected without harming the animal, the amount being gauged by the size of the body. Pearse (1909), studying the physiological effects of various substances, suggested that some of his results were influenced by the fact that the animals were spawning. Conversely, it might be that some of the shedding was induced by his treatment. For instance, he found that sodium chloride, in 10% solution, resulted in "feeding movements" and otherwise caused no harm to the animals. The same reaction was obtained this year, using 0.5 to 1.5 cc. of 10% NaCl. It seemed as if the animals might be just about to shed, but no shedding ensued. This treatment should be attempted again at the height of the spawning season. Palmer (1937) found potassium chloride a shedding stimulant in sea urchins. This was not explored carefully in *Thyone* and should also be repeated earlier in the season. However, injections of KCl did not seem to be followed by the "feeding movements" that followed the NaCl injections and, moreover, some animals responded to it by eviscerating.

SUMMARY

1. Spawning occurred in the sea-cucumber, *Thyone briareus,* during the month of June, 1948. The phenomenon appeared to wane as the month progressed. Probably early June and possibly even earlier months are the best time to find high percentages of ripe germ cells.

2. The process of shedding is described, together with various details concerning the handling of shedding animals and their gametes.

3. The following possible factors in shedding are discussed: Time of day, amount of light, presence of a shedding male to induce shedding by the female, size, quantity of sea water, freshness of animals, temperature, etc. It is shown that the first three are not essential prerequisites for shedding.

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THE PHYSIOLOGY OF EXCRETION IN MOLGULA (TUNICATA, ASCIDIACEA)

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INTRODUCTION

Twelve years ago (Das, 1936) the author wrote: "The excretory function in ascidians is attributed to a number of organs, the renal nature of some of which is still imperfectly understood. The refringent organ of the Botryllidae, the pyloric gland of the Cynthiidae, the renal vesicles of the Molgulidae and Ascidiidae, the parietal vesicles of the Cynthiidae (Pyuridae), and the neural gland of all ascidians, have been described by different workers as excretory organs." Although our knowledge of the subject has advanced considerably due to the contributions of Azema (1926, 1928, and 1929) and George (1936), so far as the fundamentals of the subject are concerned the above mentioned remarks still hold true.

The present investigation was taken up by the author during his stay in Woods Hole, in the summer of 1947, with a view to elucidating the structure, nature and relationships of the excretory organs and their products in *Molgula manhattensis* DeKay.

Lacaze-Duthiers (1874, 1892) was among the very first to locate and describe the renal organs in *Tunicata* including the renal sacs in Pyuridae (Cynthiidae). Roule (1885) described renal organs in Phallusidae, while Herdman (1888, 1899) described excretory organs in Ascidiidae, Botryllidae, and other ascidians; Dahlgrün (1901) worked on the excretory organs of Botryllidae and Ascidiidae, and compiled an account of excretory organs in *Tunicata*. Das (1936) gave an account of the excretory organs in *Herdmania,* while George (1936) described blood cells bearing excretory matter in *Tunicata*.

Küpffer (1872, 1874) was the first to demonstrate uric acid in *Tunicata*. Sulima (1914) attacked the problem of physiology of excretion in ascidians and showed that uric acid and other purine bases were the chief constituents of the excretory products. Schmidt (1924) confirmed the presence of birefringent purine derivative granules in the renal concretions of ascidians. Millot (1923) extended the investigation to other ascidians, and not only found purine bases as the chief excretory product, but discovered that in Pyuridae (Cynthiidae) the excretory granules are always made up of xanthine. Azema (1926–29) in a series of papers dealt with the mechanism of excretion in some ascidians, and demonstrated the presence of purine bases as granules inside certain blood and connective tissue cells. Finally, George (1936) has shown that certain blood cells have refractory granules of an excretory nature, and that these may circulate freely in the blood stream or be localized in the connective tissue.

From our present knowledge of the subject, therefore, *Tunciata* can be divided into two groups, based on the presence or absence of definite renal vesicles or sacs which not only filter the nitrogenous excretory products from the blood, but also store them in a more or less insoluble form as solid renal concretions. These two groups of *Tunicata* may be called:

(a) *the renal type*—with a definitive renal sac, and
(b) *the arenal type*—without any such sac.

By far the greater number of tunicate species belong to the arenal type, the renal species being mainly confined to the Ascidiidae, the Molgulidae and the Pyuridae.

In the arenal tunicates, the renal function is performed by the renal cells of the connective tissue and blood, as well as the central part of the neural gland (George, 1936; Das, 1936). The localization of the excretory granules may take place in the walls of the vas deferens (*Ciona;* Roule, 1885); the mesenchyme meshwork between oesophagus and stomach on the one hand and rectum on the other (Botryllidae; Dahlgrün, 1901); the immediate neighborhood of the gut (*Ciona intestinalis;* Dahlgrün, 1901); the white pigmented patches on siphons (*Ascidia pellucida;* Azema, 1929b).

The renal tunicates, on the other hand, have a large well-marked vesicle (Molgulidae; Roule, Lacaze-Duthiers) or a number of small vesicles or sacs arranged inside the body wall or in other regions of the body (Ascidiidae and Pyuridae; Lacaze-Duthiers, Dahlgrün, and Azema). These multicellular, blind, ductless, renal sacs not only store a fluid comparable with the urine of other chordates, but strangely enough continuously deposit inside them solid concretions that accumulate throughout life (Azema, 1926).

It is remarkable that in the same family we have some species with definite renal vesicles and others which have only scattered renal cells; e.g. in the family Pyuridae, Dahlgrün (1901) records renal vesicles in *Cynthia dura* and *Microcosmus scrotus,* while Azema (1929a) found no renal vesicles in a number of Pyurid species, but renal cells were present. Similarly George (1936) could find no renal sacs in *Pyura vittata* although renal cells were again present. Das (1936) could find no renal sacs in *Herdmania pallida* (Pyuridae) but found renal cells with refractory granules not only in the visceral region, but also being discharged into the lumen of the neural gland, whence they pass out through the neural duct into the branchial cavity and thence to the outside. According to the present state of our knowledge it is highly improbable, however, that individuals of the same species may possess both the renal vesicles as well as the localized renal cells, although scattered renal cells may often be found.

Material and Methods

The renal vesicle in the Molgulidae is a large bean-shaped sac in which waste matter is gradually deposited and stored in the form of concretions throughout the life of the animal. *Molgula manhattensis* was selected because of its abundance in the Woods Hole region, and because it is comparatively easy to expose or isolate the renal sac in the living condition. The structure of the renal sac was investigated both in the living and the fixed material, while the renal concretions were examined under reflecting as well as polarizing microscopes.

The renal fluid was extracted from living renal sacs by means of a specially made micro-pipette. Care was taken to store the renal fluid under a layer of heavy neutral mineral oil, and the method of Walker (1930 and 1937) used to determine the total molecular concentrations. The correlations between body weights and weights of renal vesicles were obtained by carefully weighing the entire live animal as well as the freshly isolated renal vesicles under standard conditions. The renal concretions were carefully separated from each vesicle, dried for a half hour at constant temperature, and weighed quickly when each batch was ready.

Structure of the Excretory Organs

The main excretory organ in *M. manhattensis* is the large, blind, and ductless renal sac situated on the right side of the body, attached to the body wall or mantle just below the pericardium. It is a thin-walled, elongated, bean-shaped sac and contains a light, yellowish-brown fluid together with the solid concretions deposited in it. It is firmly attached to the wall of the mantle and is in such close proximity to the pericardial wall anteriorly, that it is difficult to separate the renal vesicle without destroying the heart. With each wave of contraction of the heart, the wall of the pericardium is pressed against the antero-dorsal wall of the renal sac, so that the waves of contraction appear to move over this part of the sac as a series of half-ring contractions.

The renal sac lies at about two-thirds distance from the antero-dorsal border of the animal and about one-third from the postero-ventral. It lies across the mantle occupying about one third of its width at its middle (Fig. 1). In a full grown animal measuring 4 × 3 × 1.5 cm., the sac is about 16 mm. long and about 6 mm. at its greatest width. One end of the sac is bluntly rounded while the other is tapering and bent, appearing like an elongated bean in outline (Fig. 2). It is circular in cross-section in the fresh condition but tends to become flattened from side to side in fixed material (Fig. 3). In very young specimens the length of the sac is comparatively short and it appears perfectly bean-shaped; but as the animals increase in size, the sac increases more in length than in width.

The wall of the sac consists of a single layer of cells (one cell thick) which is bounded by a thin basement membrane. Each cell is almost like a cubical epithelial cell although the width is greater than the height. The nucleus lies nearer the basal end of the cell, while the cytoplasm contains one, two, or more vacuoles of different sizes (Fig. 4). The structure of each cell was best seen in the living condition, when a light methylene blue intra-vitam stain was used. The largest vacuole lying on the distal periphery of the cell always takes up the maximum stain, and contains a small mass (or two or three masses) of solid birefringent concretions that appear very much like the ones seen by George (1936) in the blood cells of *Polyandrocarpa tincta.* When kept under continuous observation, these concretions are seen to mimic "Brownian movement," increase in size, and be evacuated into the cavity of the renal sac, while a fresh vacuole soon takes the place of the one discharged. These cells are therefore primarily instrumental not only in the absorption of excretory material from the body fluid bathing the outside of the sac, but also in the isolation and final deposition of solid excretory products into the renal sac. The cavity of the sac is filled with the transparent, yellowish-brown, renal fluid.

The renal concretions lie free in the cavity of the sac. They are located in concentrated clumps or masses more at the rounded end of the sac, and spread out in a more or less continuous chain, growing towards the tapering end the the sac. The

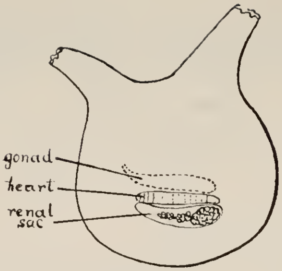

Fig. 1.

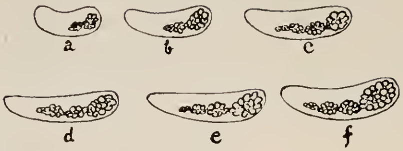

Fig. 2.

Figure 1. Diagrammatic sketch of right side of animal after removal of test to show position of renal vesicle (bottom) in relation to pericardium (middle) and right gonad (top).

Figure 2. a–f: Stages in growth of renal sac and renal concretions, (a) being the youngest and (f) full grown.

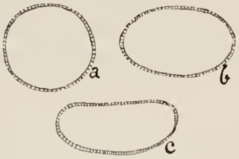

Fig. 3.

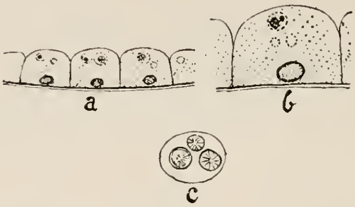

Fig. 4.

Figure 3. a and b: shape of fresh renal sac in T.S.; c: flattened outline after fixation.

Figure 4. a: the renal epithelium, × 120; b: one renal epithelial cell showing a main vacuole containing concretions, and two secondary vacuoles being formed, × 250; c: the main vacuole enlarged to show formative renal concretions, × 750.

chain can be broken up into clumps even in the living renal sac, by merely shaking it. Each clump consists of a mass of discrete rounded or oval bodies, the birefringent granules, some of which are large and others small. The smallest ones measure about 3 to 4 μ across, while some of the largest ones were 30 to 40 μ in diameter. The larger granules show definite concentric lamellae or layers of growth, the central part of the granule showing radial striations. Two, three, or more con-

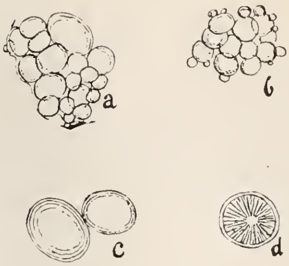

Figure 5. a and b: mass of renal concretions, showing attachment of later depositions on the previously deposited concretions in the renal sac; c: two concretion-bodies showing characteristic concentric layers; d: T.S. of a concretion-body showing radiating lines.

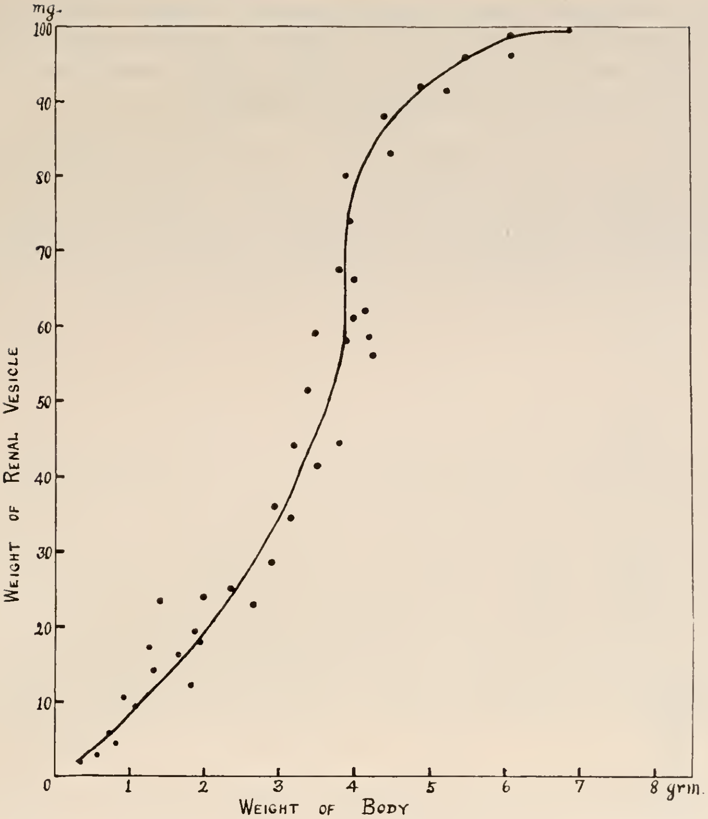

FIGURE 6. Correlation between body weight and weight of renal sac.

centric layers can be observed under the higher powers of the microscope (Fig. 5). This deposition clearly occurs from the renal fluid, without the wall of the sac taking any direct part in its formation. Smaller animals have definitely smaller excretory granules, while the largest ones show three, four, or more concentric lamellae. This appears to indicate that the rings or layers are formed by different rates of deposition of excretory matter, dependent on the differences in rates of metabolic activity at different periods in the life of the tunicate.

The neural gland opens by a neural duct into the branchial cavity, as in other tunicates. The cilia of the duct are long and lash away from the gland[1] towards the opening of the duct as seen in ducts dissected in Ringer's fluid. Cells bearing birefringent granules were seen to be extruded into the lumen of the gland and carried out by the "stream" produced by the cilia of the neural duct. This process is similar to the one observed in *Herdmania* (Das, 1936).

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[1] Huus (1937) stated that "the ciliary pit whose function is not as yet known, may probably be an organ for the reception of sexual stimuli, and that the neural gland which is closely connected with the ciliary pit, responds to these stimuli with a hormone production which in turn calls forth spawning." Similarly, Butscher (1930), and Bacq and Florkin (1935) have shown that the neural gland in ascidians produces a hormone with similar physiological effects as pituitrin. Now a hormone is always produced by a ductless gland. The neural gland has not only a duct with cilia that beat outwards, but the entire central part of the gland consists of branching ductules joining to form the main duct. The outward beating cilia would not allow passage of fluids into the gland, although the dorsal tubercle itself may receive sexual stimuli. Pituitrin is probably produced by the peripheral part of N. gland.

CORRELATION BETWEEN BODY WEIGHT AND WEIGHT OF RENAL SAC

Dahlgrün (1901), Azema (1926–29), and George (1936) state that waste matter is gradually deposited in the renal sac and stored in the form of concretions throughout life. They have not given any quantitative estimates of the concretions, nor have they established any correlations between the weight of concretions and the body weight, which alone would elucidate the rate and mode of disposal of excretory products. The present author isolated over one hundred renal sacs from animals of all possible sizes available at Woods Hole, ranging from a body weight of about half a gram to as large as seven grams. The weights of a series of individuals of graded sizes were taken and the weight of the renal sac of each was determined. The results are shown in graphical form in Figure 6. It is apparent from

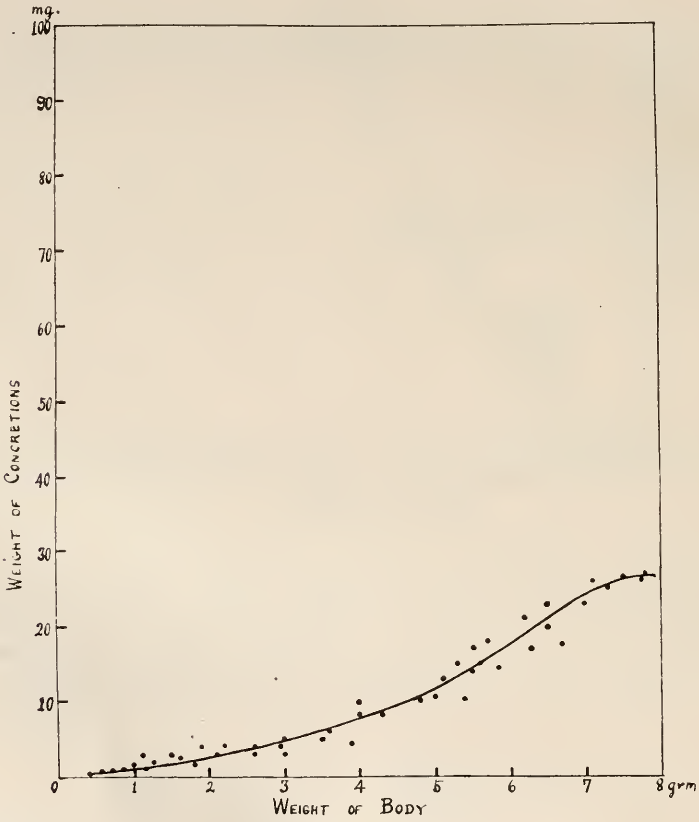

FIGURE 7. Correlation between body weight and weight of renal concretions.

the nature of the curve that the growth in weight of the renal sac is not directly proportionate to the growth in body weight. Up to a body weight of about three grams, the rate of growth of the sac is proportionate to that of the body; above this size, however, the rate of growth of the sac outstrips that of the body until a body weight of about five grams is reached. Thereafter the rate of growth of the sac becomes slower than that of the body, little growth taking place above a body weight of seven grams. This correlation may be interpreted as that between the body of the tunicate and the renal fluid, since the latter constitutes over 80 per cent by weight of the renal sac. It can thus be concluded that comparatively more renal fluid is present in the sac in half to full grown tunicates than in the younger or older stages. The correlation between body weight and weight of renal fluid is a dynamic one, as explained later.

Correlation between Body Weight and Weight of Renal Concretions

If, as found by past workers on the subject, the solid concretions in the renal sac are stored throughout life, it was surmised that there should be a straight line relationship between the body weight and the weight of renal concretions in animals of different sizes. Actually, however, the data from the present study yield a curve which is far from a straight line. From a body weight of about 0.4 gm. up to about 3 gm., the increase in weight of the concretions is rather small; but above this the weight increases rapidly and the correlation curve shows a steep ascent (Fig. 7). This means that instead of the accumulation of excretion being proportional to the body weight (i.e. instead of the increase in excretory material being proportional to the growth of the animal), the excretory material is deposited at a faster rate as the animal grows older.

Correlation between Weight of Renal Vesicle and Weight of Renal Concretions

This correlation is shown in Figure 8. It appears to be almost a straight line relationship in its first half, meaning that the rate of accumulation of concretions is directly proportional to the increase in weight of the renal sac. In other words in the first part of the life of the tunicate, the increase of renal concretions is directly proportional to the increase in renal fluid inside the sac. As the tunicate grows older, however, the rate of increase of the renal sac is overtaken by the rate of deposition of renal concretions; and we find the upper part of the correlation line curving steeply upwards (Fig. 8). This increase falls rapidly as the maximum size of the tunicate is reached; and finally the weights of both the renal sac and the concretions remain almost stationary even with an increase in body weight. The final stage, when no further accumulation of concretions is possible, may be called the *saturation stage*. The tunicate could not live much longer once this stage has been reached.

The renal sac from large living *Molgula manhattensis* was removed and the animals kept under observation for a week or more, but no regeneration of the sac or formation of secondary renal sacs was observed. This is exactly the reverse of what obtains in multivesicled animals like *Ascidia* and *Ascidiella*, where a number of renal sacs are present instead of only one as in *Molgula*. In *A. mentula* (Azema, 1926) renal sacs are formed quite early in development; but instead of all the sacs being formed simultaneously, they are formed successively in the regions around the oesophagus, stomach, and intestine. New vesicles arise as the old ones are used up. It is apparent, therefore, that these multivesicular tunicates have a more effective excretory system than the univesicular ones.

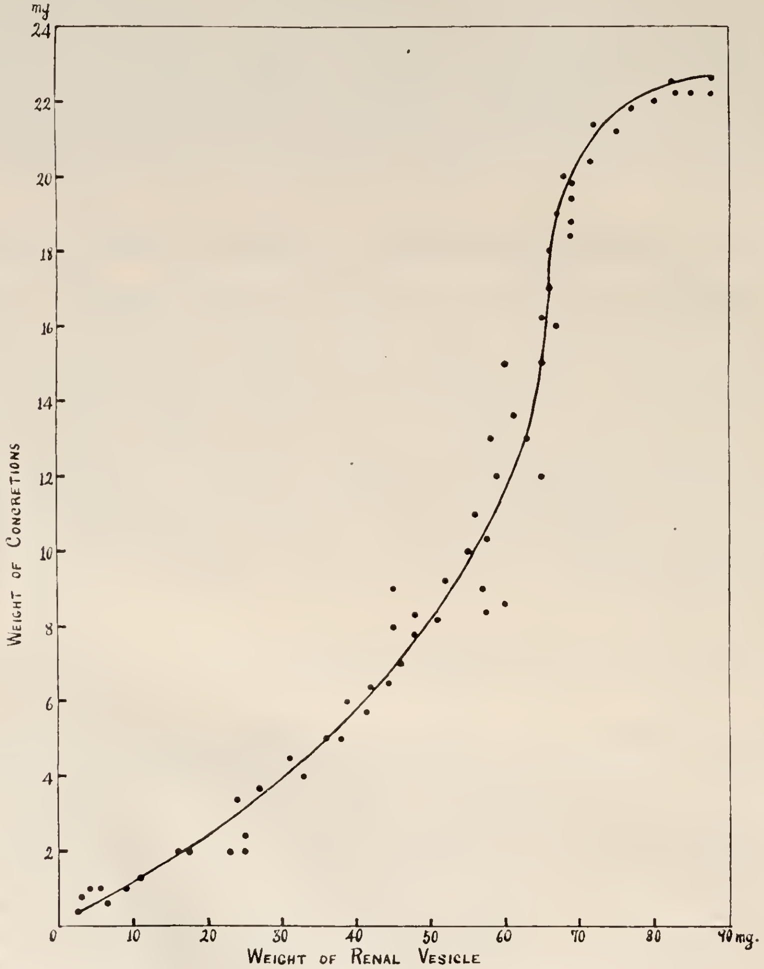

Figure 8. Correlation between weight of renal vesicle and weight of renal concretions.

The Renal Fluid

The renal fluid, when examined in fresh condition, is a clear, pale, yellowish-brown fluid which turns darker on exposure to air. The pH of this fluid ranged from 7.4 to 7.6 as determined by the Cambridge glass-electrode potentiometer. Tunicate urine is, therefore, slightly alkaline in nature. That this is caused by free ammonia was determined by the permutit method. No urea was present in any of the renal fluid samples. A full quantitative estimation of all the constituents of the fluid could not be undertaken in the short time available at Woods Hole, but will no doubt be attempted in the near future.

Total molecular concentration and tonicity

Pure samples of the renal fluid were obtained from freshly dissected-out renal sacs by inserting a chemically clean, dry, glass micropipette into several sacs in turn. The pooled renal fluid was collected under mineral oil in order to avoid evaporation, absorption of water, or any changes due to exposure in air. The total molecular concentration of these fluids was compared with that of known NaCl solutions in "Barger tubes" (glass capillary tubes) using the method of Walker (1930 and 1937). This method involves sealing a series of micro-drops of the two fluids to be compared, each separated from its neighbours by a tiny air-gap, into chemically clean, dry, glass capillaries of very uniform internal diameter. Relative volumes of the drops are determined by measuring the lengths of the columns in the capillaries under a compound microscope, using a filar micrometer. These measurements are repeated at intervals for a period of two or three days, the capillaries being stored in a water bath between times. Consistent lengthening of a drop and shortening of its neighbours, indicates its higher total molecular concentration and vice versa. Thus by using saline solutions of known strength, the molecular concentration of the unknown fluid can be determined. Microdrops were prepared and read for:

(a) renal fluid against known saline solution
(b) body fluid against known saline solution
(c) sea water against known saline solution
(d) renal fluid against sea water
(e) body fluid against sea water

The results may be summarized in tabular form:

SPECIMEN	APPROX. CORR. NaCL CONC.
Pooled body fluid	2.75 gm./100 cc.
Sea water	3.10 gm./100 cc.
Pooled renal fluid	3.45 gm./100 cc.
One sample of renal fluid	Hypertonic to sea water

This comparison of the total molecular concentration of the renal fluid, the sea water, and the body fluid, has, as will be presently seen, led to very interesting results. The renal fluid was thus found to be *hypertonic* to sea water in which the animal lives, while the body fluid was *hypotonic* to sea water, i.e.,

$$\text{renal fluid} > \text{sea water} > \text{body fluid.}$$

Osmoregulation

The occurrence of hypertonic urine in *Tunicata* throws new light on osmoregulation in this group, little work on this subject having been done in the past. The body of the tunicate is closed to all exchanges between the surface and the sea water due to the covering of the test. But the current of sea water passing through the branchial siphon into the pharynx and out through the atrial siphon, brings the extensive branchial wall and vessels into close contact with it. The O_2 is absorbed and CO_2 is thus excreted through the branchial vessels. The body fluid, as established above, is *hypotonic* to sea water; and thus it is apparent that an osmotic gradient is maintained in the tunicate between the internal and the external environment. Whether water required by the animal is taken from the sea water into its alimentary canal like the marine teleostean fishes, is not definitely established but is quite probable. The role of the hypertonic renal fluid is, however, quite definite. There is a constant flow of fluids from the blood into the renal sac, the excretory products being extracted mainly by the cells constituting the wall of the sac. The renal cells not only maintain an active osmotic gradient, but allow more or less water to pass out of the sac according to the requirements of the animal. Experimental animals were kept in grades of sea water ranging from 40 per cent to 80 per cent (hypotonic), and from 110 per cent to 160 per cent (hypertonic), for a fixed period of five hours each. Each animal was weighed and a "twin" found weighing about the same. The "twins" were kept as controls in 100 per cent sea water for the same period. The renal sacs of each living experimental animal and its "twin" were rapidly dissected out and both weighed against each other. It was observed that renal vesicles from animals in diluted sea water weighed more than their controls, and that the extra weight was inversely proportional to the concentration of sea water in which the animals were kept; that is, the lower the concentration of sea water, the higher the weight of the renal sac. On the other hand, renal vesicles from animals kept in concentrated sea water weighed less than their controls; that is, the higher the concentration of sea water, the lower was the weight of the sac

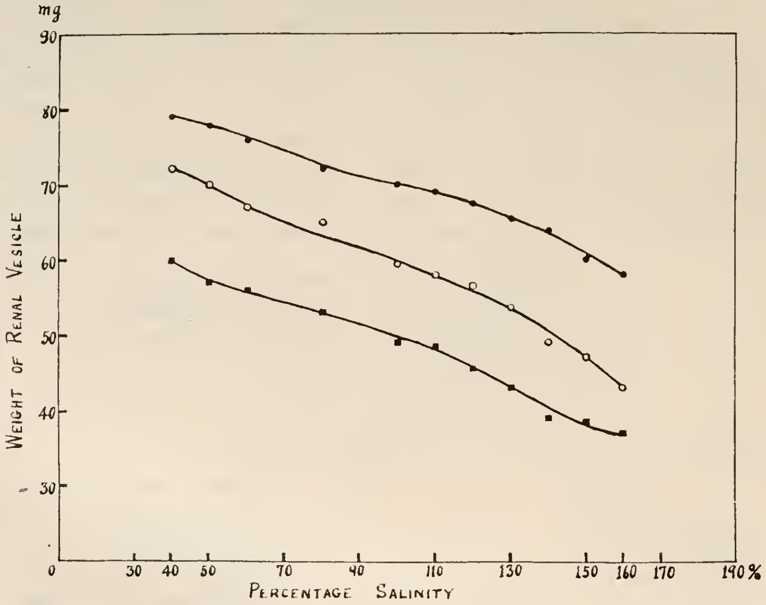

Figure 9. Correlation between weight of renal sac and different concentrations of sea water. Three experimental series are represented, the animals in each series weighing about the same.

(Fig. 9). It can therefore be concluded that the renal sac is an active osmoregulatory organ as well as an efficient extractor and storer of excretory material.

THE RENAL CONCRETIONS

As stated above, the concretions are dark brown in color, are birefringent in polarized light, and acquire a yellowish tinge by reflected light when spread out thinly on a glass slide. The concretions are insoluble in water and in dilute acids, but easily dissolved in diluted alkalies (NaOH, KOH). A qualitative analysis of the concretions was made to test for uric acid, xanthine, guanine, adenine, and creatinine. Folin's test yielded faint traces of creatinine; the murexide test and microscopic examination showed that a part of the concretions was made up of uric acid; Weidel's reaction indicated the presence of moderate amounts of xanthine; while the picrate test showed that even guanine was present. The zinc test for adenine was entirely negative, and the presence of hypoxanthine was also extremely doubtful. No allantoin could be traced in the concretions.

As shown in Figure 8, the rate of accumulation of concretions is directly proportional to increase in weight of the renal sac in the young tunicate. As it grows older, however, the rate of increase of the renal sac is overtaken by the rate of deposition of the concretions; and finally, when the saturation stage is reached, no more can be deposited.

THE MECHANISM OF EXCRETION

According to the findings of Küpffer (1872, 1874), Sulima (1914), Millot (1923), Schmidt (1924), and Azema (1926–1929), the main excretory products are: uric acid in some Ascidiidae, xanthine in Pyuridae, and un-named purine derivatives in other ascidians. This picture becomes more confusing if we now add guanine (found in Molgulidae) to this list. In all animals the ultimate source of the excretory nitrogen derivatives are the α-amino-N and the nucleoproteins. A list of the main nitrogen end products of various chordates may be given here:

sharks, dogfish—urea
bony fishes (Teolosts)—ammonia
frogs, newts—urea
turtles—urea
snakes, lizards—uric acid
birds—uric acid
mammals—urea

In tunicates, however, it appears that different families have, as the main nitrogen end product of excretion, either uric acid or one of the purine derivatives.

In chordates, where we find uric acid, urea, or ammonia as the main nitrogen end product, the conversions stop at these respective steps. In the tunicate, although some of the nitrogenous matter gets converted into uric acid, a large part of it appears to stay as xanthine and guanine. We thus find in *Tunicata* a unique case of incomplete conversion, which accounts for the presence of xanthine and guanine, as well as uric acid. The author hopes to make exact quantitative estimations of these three constituents, in different tunicates, in the near future.

SUMMARY

1. The present contribution includes a review of past work on excretion in *Tunicata.*

2. In *M. manhattensis* the single-celled wall of the renal sac absorbs the excretory products, develops the concretions, and discharges them into the cavity of the sac, where they are stored throughout life.

3. The concretions consist of granules, some of which show three or four concentric lamellae caused by further deposition of excretory matter on top of the originally secreted granule.

4. The correlation between body weight, weight of renal sac, and weight of concretions is given. It is shown that accumulation of concretions is not uniformly maintained throughout the life of the tunicate.

5. If the adult renal sac be entirely removed, no regeneration or formation of secondary sacs takes place.

6. The renal fluid is *hypertonic* to sea water, whereas the body fluid is *hypotonic.*

7. The molecular concentration of the renal fluid is 3.45 gm./100cc.; of the sea water at Woods Hole 3.10 gm./100 cc.; and of the body fluid 2.75 gm./100 cc., corresponding to NaCl concentrations.

8. The renal concretions contain xanthine, guanine, and uric acid.

ACKNOWLEDGMENT

I am indebted to Dr. Parpart, director of the physiology course at Woods Hole during 1947, for allowing me a table in the physiology laboratory and giving me facilities for work. My thanks are due to Dr. Kempton, professor in charge of the section on excretion and osmoregulation, for his kind advice during the course of the investigation. I should like to express my gratitude to Dr. Kellogg, my colleague in the physiology course, for confirmation of my finding, arrived at by experiments on osmoregulation, that the renal fluid is hypertonic to sea water; and also for estimation of the total molecular concentrations of renal fluid, sea water, and body fluid of *Molgula manhattensis* by the "Barger tube" method. Finally, I must thank the staff of the Woods Hole Laboratory for supplying animals when required and for the all-round kindness shown me during my stay in the summer of 1947.

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THE LIFE HISTORY AND BIOLOGY OF A MARINE HARPACTICOID COPEPOD, TISBE FURCATA (BAIRD)[1]

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Introduction

Only a few free-living marine copepods have been reared with a view to determining the details of their complete life histories. This can no doubt be attributed to the difficulty experienced in culturing most species through the entire life cycle. Considerable literature has accumulated on the morphology of developmental stages and on certain aspects of the biology, particularly of the more important planktonic calanoid species, especially *Calanus finmarchicus.* This species has also been reared through its entire life cycle in cultures (Lebour, 1916), and its biology studied under laboratory conditions more recently by Raymont and Gross (1942). The cyclopoid, *Oithonina nana,* has also been reared through the developmental stages (Murphy, 1923). But pertinent questions relative to the age at maturity, fecundity, and life span of individuals have received scant attention for other marine species. The fresh-water copepods on the other hand have been the subject of a large amount of investigation and much detail is known about their life histories and reproduction (see especially Gurney, 1931; Ewers, 1930, 1936).

Of the marine harpacticoids, *Longipedia coronata* Claus, *L. scotti* G. O. Sars, and *L. minor* T. A. Scott have been reared sufficiently to reveal specially significant aspects of their biology (A. G. Nicholls, 1935). *Tigriopus fulvus* (Fischer) has also been studied in considerable detail by Fraser (1936) and Shaw (1938). The morphology of the larval stages of a number of other marine harpacticoids has been described by various investigators, especially Chappuis (1916), Brian (1919, 1922), Gurney (1930, 1932), and Nicholls (1941). Many of the littoral species, though less conspicuous than planktonic copepods, fill an important niche as microscavengers on the bottom, and therefore warrant a close study for ecological reasons.

Tisbe furcata, also known under the generic names of *Idya* and *Idyae* (see Wilson, 1932), is a littoral cosmopolitan species. It thrives in cultures and therefore often occurs as a contaminant in laboratory cultures of other organisms. At Scripps Institution it is constantly present in the salt-water system, aquariums, etc., to which it gains entrance from the sea through the pumping system.

In 1934 it occurred in such numbers that it contributed materially to formation of flocculent detritus in the pipes. This detritus when matted together with loosened calcareous tubes of *Spirorbis* (also established in the system) caused serious clogging of water delivery jets in the aquariums.

There is some possibility that "culture forms," characterized by small differences in proportions of appendage segments and strength of setae, may develop in *Tisbe* in these cases of isolation or semi-isolation from the "wild" population. However,

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[1] Contributions from Scripps Institution of Oceanography, New Series, No. 399.

Monk (1941) considers the present form to be a new variety, *johnsoni*. Controlled tests were not made of the factors involved in development of culture forms, but the temperature range (about 17–21° C.) over which the animals were reared is small compared to that used by Coker (1934) in experiments affecting the form of fresh-water *Cyclops*.

In nature *Tisbe furcata* is usually found in shallow water among algae or on the bottom where it is frequently the dominant species, but being capable of swimming, it is sometimes found also in the coastal plankton. Sars (1903–1911), who has given a full description of the adult, found it to be the most widespread of the harpacticoids in Norway wherefrom he also reports a large deep-water form of the species.

THE DEVELOPMENTAL STAGES

During its life cycle *Tisbe furcata* passes through six naupliar and six copepodid stages, the last of which is the adult. The female is about 1.0 mm. and male 0.7 mm. long. The instars are separated by only one molt as in other copepods that have been studied. The female carries the eggs in a single egg-sac attached to the genital segment. The early cleavage and embryological stages have been studied by Witschi (1934).

All drawings have been made from specimens reared in the culture experiments to be described later. The assistance of Dr. Cecil R. Monk is gratefully acknowledged in connection with the 1938 cultures and with the developmental stages.

Naupliar Stages

(Plate I, Figs. 1 to 6)

In all these stages the larvae are normally benthonic, but occasionally they are taken in the plankton near shore where they have been swept off the bottom by water currents. They are colorless, subcircular in outline and possess a small red eye spot.

Nauplius Stage I (Fig. 1).—Average length 0.062 mm.; labrum circular flap; first antennae three-segmented, the distal segment with three terminal setae; the second antennae strongly built, the endopod terminating in a prehensile hook, exopod normal. The first basis of the second antennae is provided with a large but rudimentary masticatory blade or hook with a small seta at its base, and the second basis bears two strong spines. The mandibular palp possesses a splender exopod of three indistinct segments, the distal segment bearing one short and one very long seta; the endopod bears two strong short hooks and two setae. The caudal armature consists of two rather long flaccid setae.

Nauplius Stage II (Fig. 2).—Average length 0.082 mm. The obvious structural advancements over Stage I consist of increase in strength of the masticatory blade of the second antennae and the appearance of two strong setae, one each on either side of the posterior ventral side. These setae are the earliest visible fundaments of the first maxillae. This early appearance of the maxillae is characteristic of the harpacticoids. In the strange nauplius larvae of *Longipedia* the first maxillae are present already in the first stage (Nicholls, 1935). In this respect the development of harpacticoids is similar to that of many cyclopoids. In calanoids the rudiments of these appendages first appear in the third or fourth nauplius stage.

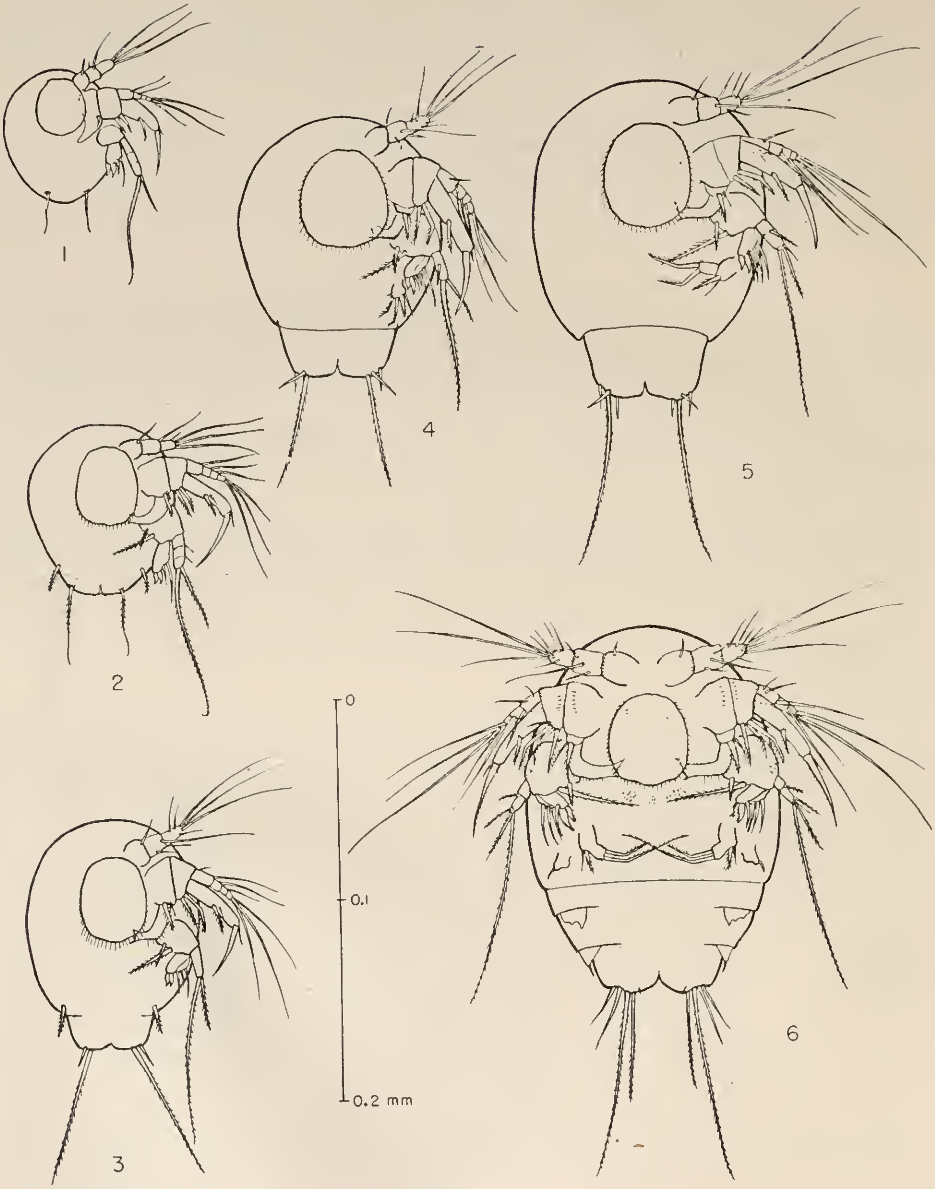

Plate I

Development of *Tisbe furcata*

Figures 1–6. Nauplius stages I to VI—ventral; all drawn to same scale with aid of camera lucida.

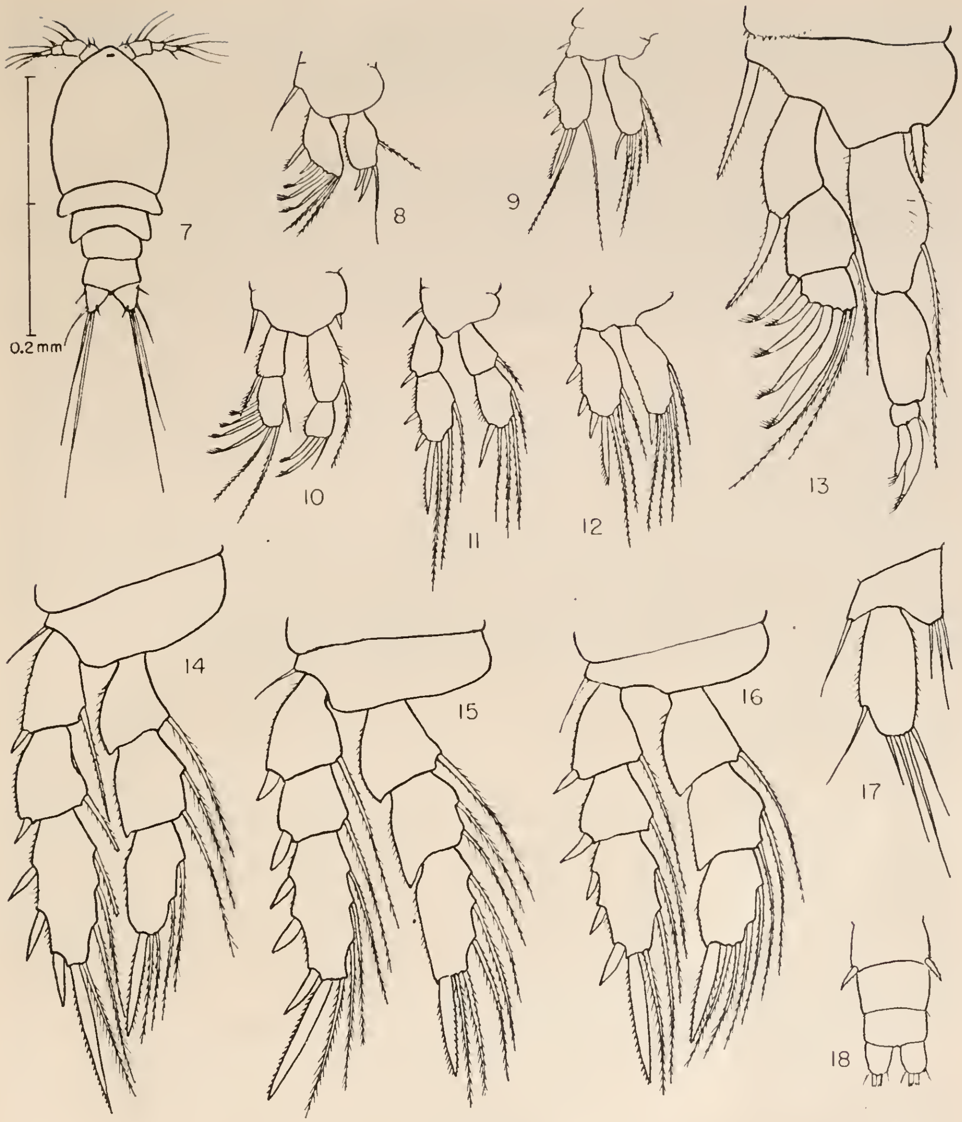

Plate II

Development of *Tisbe furcata*

(*Camera lucida* drawings)

Figure 7. Copepodid stage I—dorsal.

Figures 8 and 9. First and second feet—Copepodid stage I.

Figures 10, 11, and 12. First, second and third feet—Copepodid stage II.

Figures 13, 14, 15, 16, and 17. First, second, third, fourth, and fifth feet—Copepodid stage V—female.

Figure 18. Posterior ventral portion of urosome of male copepodid stage IV to show sixth feet as represented by spines.

Nauplius Stage III (Fig. 3).—Average length 0.110 mm. As in II, but with three marginal setae on the distal segment of the first antennae, one additional seta on the endopod of the mandibular palp, and one short seta issuing from the outer angle of each caudal ramus. The original caudal setae are now much longer and more rigid than before.

Nauplius Stage IV (Fig. 4).—Average length 0.130 mm. As in III, but posterior segment of body more clearly defined. The distal segment of first antennae with increased number of marginal setae that can be discerned only with difficulty and are therefore not useful in identification as in calanoid larvae. The endopod of mandibular palp with four setae; the first maxillae, each showing one segment with three spines and a long seta; each caudal ramus with two short outer setae at the base of the long seta.

Nauplius Stage V (Fig. 5).—Average length 0.156 mm. As in IV, but the rudimentary first maxillae have two segments each, the distal segment with two strong curved spines and the proximal with one spine and one seta. Caudal ramus each with an additional short seta set at the inner base of the long seta making a total of three short setae.

Nauplius Stage VI (Fig. 6).—Average length 0.178 mm. As in V, but distal segment of first antenna with five marginal setae; one-segmented rudiment of second maxilla is evident with one short seta; rudiments of first and second pairs of legs present but the maxillipeds were not evident. Caudal armature as in V, but the formerly short setae are much longer, especially the inner pair.

Copepodid Stages

(Plate II, Figs. 7 to 18)

The sixth nauplius stage metamorphoses to the first of six copepodid stages, the last of which is the adult. The period required to pass from hatching of the first nauplius to the last copepodid is about 16 days at temperatures of 17° to 21° C.

The first copepodid stage is 0.20 mm. long and resembles the adult in general appearance, but there are present only the first two pairs of feet with a rudiment of the third pair and the metasome and urosome have respectively only three and two divisions, using the terminology of Sars. In subsequent stages one additional pair of feet is added for each molt except the fifth.

The order of segmentation of the feet follows the same pattern as that found in calanoid copepods. Each pair of feet at the time of its appearance possesses only one segment in each ramus, and in the next following molt each ramus becomes two-segmented and remains thus until the fifth molt (Copepodid V) when the rami of all the feet, 1 to 4, acquire three segments each (Plate II, Figs. 13–16). The new segment is derived from the proximal portion of the distal segment. This is illustrated in the first pair of feet which already in the fifth copepodid stage furnish diagnostic features of the genus. The fifth pair of feet which furnish the specific characters are not completely diagnostic until the sixth copepodid stage.

The sexes are readily separable morphologically in the fourth copepodid stage. The male in this stage is not only smaller in body size but the sixth pair of feet is already clearly developed, each foot represented by a small spine (Plate II, Fig. 18).

In the female these feet are wanting or indistinguishable from the row of fine spines on the urosome.

The Biology of Tisbe Furcata

Culture experiments

Syracuse watch glasses and low stender dishes of 50 ml. capacity were used for culture dishes in order to facilitate direct examination under the low-power microscope. The water used in the dishes was filtered to eliminate the possibility of contamination with foreign eggs or nauplii from the general sea-water supply. A fairly constant temperature was maintained by keeping the culture dishes on a table of running sea water, though the animals thrive in dishes kept at ordinary room temperatures.

A variety of marine foods was tested including ground dried mussels and scallops, dehydrated kelp, fresh seaweed and fecal pellets of *Nereis*. Each of these given individually in very small amounts was quite satisfactory as food. Shavings of fresh brown seaweed or dehydrated kelp appeared to be the best single food. Fresh seaweed has the disadvantage that the slime secreted tends to entrap the organisms. In general, however, the cultures were fed various mixtures of the above items so that some measure of selection was possible for the animals. About twice a week the dishes were cleaned by removing the debris and about one half of the water with a pipette, and a fresh supply of water and food added.

Life history series were obtained in four separate years, i.e., March-May 1935, June-July 1938, April-June 1942, and Sept.-Dec. 1947. During this time 30 or more broods and some of their progeny for two generations were studied more or less completely. During these periods no marked seasonal changes were observed in the breeding habits, or rate of reproduction, though a somewhat higher average number of eggs per egg-sac was noted in December.

Since observations on the 1942 series were the most continuous, they will form the main basis for discussing the salient features in the biology and life cycle of this species. Where data can be drawn from the earlier or later cultures, they will be made a part of the summary of time involved in the various phases or periods of the life cycle.

The lapse of time given for the various periods is the minimum time, or the average of several observed minima. For example, the first appearance of a nauplius in a culture was set as the beginning of the naupliar period and the first appearance of a copepodid marked the end of the naupliar period for any one brood.

Number of eggs produced

In order to obtain an estimate of the average number of eggs produced by each female per brood, counts were made of the eggs in the brood sacs of ten ovigerous females selected at random from the general population in March 1942. The number ranged from 29 to 82, with an average of 42.9 eggs per sac. There is obviously a very wide range in the number of eggs produced per egg-sac depending upon how long the individual has been laying eggs, and perhaps also upon the season. A check count was made in December 1947 in which the numbers ranged from 45 to 93 with an average of 72 eggs in each of 12 egg-sacs. Kunz (1935) reports 30 to 41 eggs for the species in Kiel Bay.

A group of ten ovigerous females was selected at random from the same population from which the 1942 egg counts were made and isolated for experimental culture, two females per dish. Each female was removed from the dish as soon as her eggs had all hatched. Thus each of the five culture dishes contained the nauplii from two egg-sacs. Careful account was kept of each population. As they matured and the adults mated, each mating pair was removed, recorded, and placed in another dish. In this way an accurate census was obtained.

Success of hatching and survival

From the ten broods 343 individual animals reached maturity. This indicates about 80 per cent survival, if we assume that the average egg-sac contains 43 eggs.

Incubation period

The duration of the incubation period was approximated by noting the first appearance of the egg-sac and the subsequent hatching of the nauplius larvae from the sac. The minima obtained ranged from two to four days. On the basis of the average of 21 cases the period was 2.5 days. Usually the cultures were examined every one to three days.

Naupliar period

The time required for completion of the separate stages was not studied. During the naupliar stages the animals creep about actively over the bottom or cling to detritus, etc., by means of prehensile hooks on the second antennae (Plate I, Figs. 1–6). The duration of this period is from three to eight days, with an average minimum of five days based on 22 brood histories.

Sex ratios

Of the 343 individuals reaching maturity 114 or only 33.3 per cent were females. One culture dish produced 34 females and 33 males, but in all of the rest the males were in great majority. In order to compare this unequal sex ratio in the culture dishes with that of the general population an examination was made of 44 adults from the water system. Of these only 41 per cent were females. Additional analysis involving 98 specimens reared in December 1947 also showed a ratio of one female to two males.

The numerical dominance of males is of considerable interest since usually in other species of copepods the females are the more numerous, sometimes overwhelmingly so. This inequality of the sexes is often seasonal, but no seasonality has come to light in the study of *Tisbe*.

In a study of the tide pool harpacticoid *Tigriopus fulvus,* Tsen-Hwang Shaw (1938) found 58.8 per cent females in a collection of 318 specimens.

Sexual maturity

A study of the rate at which sexual maturity was attained by 87 pairs of the 343 individuals hatched from the ten egg-sacs mentioned above, showed that two pairs began mating (as indicated by clasping) just 14 days after they had hatched. The last four pairs mated in 25 days. The average time required to reach mating maturity after hatching was 16 days. The minimum period observed in earlier experiments at Scripps Institution was ten days. The period of clasping varied from a few hours up to one or more days, but there was no evidence that the actual transfer of the spermatophore to the female is delayed until she molts to the last or sixth copepodid stage. Clasping was rarely observed before the female had reached the sixth copepodid stage, and on only one occasion was there evidence of molting during the clasping period. This point deserves further study. Williams (1907) found that for the harpacticoids, *Harpacticus uniremis, H. gracilis,* and *Tachidus littoralis,* "every successful copulation must be prolonged until the female molts."

Length of generation

The lapse of time between the hatching of one generation and the first appearance (hatching) of the next generation resulting from the above matings varied from 17 to 24 days at temperatures of 17° to 18° C. during the spring months. The maximum period observed in one case was 31 days. In 1935, two individuals required 20 days and one individual only 16 days. MacGinitie (1937) in an incidental statement records that this species completes its life cycle in ten days at Corona del Mar, California.

The time required for *Tisbe* to develop from egg to production of eggs is comparable to that reported for various species of the freshwater cyclopoid *Cyclops*. Ewers (1936), for example, found that for 12 species under observation the time varied from 8 to 50 days.

It appears that *Tisbe furcata* may complete its life cycle in about one-half the time required for *Tigriopus fulvus* which under favorable conditions in the laboratory at University College of Hull required about two months (Fraser, 1936). However Tsen-Hwang Shaw (1938) states that for this species at Pacific Grove, California, the adult stage is reached in about one month. It is probable that the Pacific Grove species is distinct from *T. fulvus,* since Monk (1941) mentions only *T. californicus* from the coast.

Number of broods produced

In order to determine the approximate number of broods produced by a single female, four of the above 87 pairs in copula were isolated in individual dishes. Several additional males were added to each dish to afford ample opportunity for additional matings, and the males were replaced from time to time with younger mature males. (Lowndes, 1933, and Fraser, 1936, infer that the presence of males stimulates egg production.) Nauplii were removed from the dishes as soon as they appeared. Records were kept of the number of egg-sacs produced by each female under these conditions. One female produced 12 broods between May 9 and June 10. Two other females each produced nine broods in the same period and one produced five broods in 20 days before dying, apparently prematurely. The first egg-sacs produced were the largest, and the last two or three produced were about half the size of the first. The egg-sacs appeared with regularity, two to five days elapsing between hatchings with three days as the average period.

BIOLOGICAL

Although males were kept in the presence of the females during the whole period, only one mating was observed to take place and this preceded the entire period of egg laying. In order to test further this observation in a more crowded condition as often occurs in nature, four separate cultures were set up each containing from 8 to 20 pairs of newly matured adults in copula. A total of 54 pairs were involved. All pairs in any one dish were from the same brood and therefore of the same age. At two day intervals all adults were transferred to fresh culture dishes so that their generation could be kept separate from the younger generation constantly being produced. These adults were maintained from May 12 to June 12, and during the time following the separation of the pairs originally mated, only one instance of a second clasping was observed. This instance occurred 21 days after the original mating, and it is not known if a spermatophore was transferred. The pair concerned was isolated from the rest of the culture in order to determine if the female would continue to produce eggs for a longer time than the other females as a result of this second mating. However, no additional eggs were carried by her. This is in agreement with the earlier observations where in one case a succession of seven broods was produced by a female that had been reared in culture and isolated following one mating. She lived 70 or 71 days but produced no eggs after the 53rd day. In another test 11 ovigerous females isolated from the general population produced two to five broods, but it is not known if previous broods had been produced before isolation. Nicholls (1935) has reported a case in which a female specimen of *Longipedia scotti* had produced at least nine broods during a period of isolation from contacts with males.

The question naturally arises as to whether or not the male of *Tisbe* produces a succession of spermatophores and remains fertile after the first mating. To test this, a single male was isolated from a group of newly matured virgin males. Several virgin females were added to the dish at intervals, and records were kept of the fertility of eggs subsequently produced by each female. Out of a total of 13 virgin females brought into contact with this one male, six produced fertile eggs which hatched. The other seven females produced eggs which failed to hatch. In another test one male fertilized eight females which produced fertile eggs.

Virgin females kept in isolation have produced full egg-sacs, but in no instance was there any evidence of parthenogenesis as reported by Roy (1931) for the harpacticoid *Canthocamptus bidens*. Pine (1934) has reported parthenogenetic reproduction in *Cyclops viridis*. This, however, appears to be a misinterpretation resulting from failure to note that several egg-sacs with fertile eggs may be produced following only one mating. The females with which she worked were doubtless already fertile since they were isolated in the gravid condition from the wild.

Discussion

Rather little is known regarding the kinds of predators that feed directly upon the littoral copepods. That this type of animal food constitutes an appreciable source of nourishment for littoral animals is suggested by the fact that only a moderate population of *Tisbe* has been observed in the sea despite a relatively rapid and steady rate of replenishment as suggested by the present study. Being detritus feeders with a wide range of acceptable food, it is probable that in favorable habitats *Tisbe* population density is controlled largely by predators. Among these predators are no doubt small fish capable of capturing small prey of this kind by pursuing and picking up the copepodid stages individually, and coelenterates having tentacles supplied with nematocysts and viscid surfaces that enable capture by chance contact. Various detritus feeders scooping up particles of detritus would consume together with this many *Tisbe,* especially the nauplii, that are prone to cling to particles upon which they are feeding.

From the present study it appears that an average of about 513 eggs is produced by each female in her lifetime. This is calculated from an average of 57 eggs per brood (using the averages of March and December) and a total of nine broods. For unknown reasons only about 80 per cent of the larvae hatched in the cultures survived to adult state. Thus, using the above figure for one female, 410 survive, of which only about 135 (approximately one third) are females. Considering a succession of generations each 25 days, a prodigious number of progeny is possible, for in about 100 days (fourth generation) over 1 billion 55 million individuals of both sexes would be produced. Ten males plus 5 females make up a mass of about 1 mm.^{3}. Hence in 100 days 70 liters of copepods would have been produced.

TABLE I

Potential rate of reproduction in Tisbe furcata and Calanus finmarchicus in cultures

	No. of broods or spawnings	No. of eggs per brood or spawning	Total no. of eggs in life-time of one ♀	Interval between generations	Survival to adult
Tisbe	7–12 average 9	29–93 average 57	513	15–31 days usually 19–24 days	80%
Calanus	1–3 (4?)	1–120 usually 15–70	Maximum observed 120	Minimum 41 days	?

These figures can of course have no significance other than to emphasize that the animal substance which *Tisbe* could supply is considerable and that a very large number of copepods must be consumed regularly by enemy predators in order to keep a balance between consumption and reproduction.

It is instructive to compare certain vital aspects of the life history of this important littoral species with similar aspects of *Calanus finmarchicus,* the most widely studied of planktonic-copepod species.

In view of the vast swarms of *Calanus* which sometimes occur in the sea, one might expect that studies of its rate of reproduction would reveal aspects suggesting possibilities of greater individual fecundity than occurs in *Tisbe,* a seemingly less abundant species. In no instance, however, is a greater rate of reproduction shown for *Calanus* on the basis of studies that have thus far been made in cultures.

Table I compiled mainly from Nicholls (1933), Clarke and Zinn (1937) and Raymont and Gross (1942) shows the great discrepancies that are brought out when comparing these two genera of widely different ecological habits.

The life span of *Calanus* is greater than that of *Tisbe,* but this appears to have no bearing on the fecundity since the number of spawnings is not shown to be affected thereby. Raymont and Gross found that under laboratory conditions *Calanus* was capable of spawning throughout the year, similar to our observations for *Tisbe,* but field observations by numerous investigators show that the rate of reproduction for *Calanus* in the sea varies greatly with season, there being a marked minimum in autumn and winter when the animals survive in copepodid Stage V. No similar field data are available for *Tisbe.*

The usually great preponderance of females over males in *Calanus* should enhance the reproductive rate provided this preponderance obtains also for the hatching eggs and provided further that there are always sufficient males in the population. That the latter is probably true is indicated by the study of Gibbons (1933). There is some evidence that in *Calanus,* as in *Tisbe,* one mating may suffice for continued spawning, since females have been observed to produce fertile eggs three or four weeks after isolation.

The greater ratio of males to females in *Tisbe* seems to have no logical explanation, since it is shown that one mating suffices for all the broods the female can produce, and each male produces a sufficient number of spermatophores to fertilize seven or eight females.

In considering this anomalous situation with respect to the relative reproductive rate of the two genera in cultures contrasted with their abundance in nature, the answer might be found in the greater ease with which *Tisbe* can be reared in culture. Hence, a truer picture might be obtained for that genus, but one that is difficult to check in the field. It is probable that in nature *Calanus* actually reaches reproductive maturity at a younger age and produces more eggs than have thus far been shown in culture experiments or deduced from field observations. An obvious alternative is that the survival rate is greater for *Calanus* (possibly because of greater opportunity for dispersal both vertically and laterally). The conclusion must then be that *Tisbe* is heavily preyed upon in its natural habitat.

SUMMARY

1. *Tisbe furcata* is a littoral copepod that commonly invades salt-water systems connected with the sea. It is readily reared through all of its developmental stages. Being a scavenger, it thrives on various types of food, but thin slices of fresh seaweed and dehydrated kelp were especially acceptable.

2. Following the egg, there are six naupliar and six copepodid stages, the last of which is the adult. Each stage is separated by one molt.

3. The incubation period is from two to four days, usually about 2.5 days.

4. The total duration of the naupliar stage is three to eight days, usually about five days.

5. The first indication of sexual maturity as shown by clasping by the male occurred between the 10th and 25th days, usually about the 16th day.

6. The minimum time between generations (i.e., from egg to egg production) was 15 days, but usually between 19 to 24 days.

7. The span of life of individuals varied greatly. It was studied mainly in the females, some individuals of which lived for 40 to 50 days. The oldest specimen had a life span of 70 or 71 days, but no eggs were produced after the 53rd day.

8. The number of broods indicated by egg-sacs produced by isolated females varied from 7 to 12, with an average of about 9. Following the first egg sac, the subsequent ones appeared at intervals of two to five days, usually about three days.

9. The number of eggs in a brood varied from 29 to 93 with an average of 43 in one sampling and 72 in another.

10. Each female mated but once, and this mating sufficed for fertilization of all of the eggs to be produced. Males were capable of several matings.

11. About 80 per cent of the larvae hatched survived to adult state.

12. There is no evidence of parthenogenesis.

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FURTHER CHEMICAL ASPECTS OF THE SENSITIZATION AND ACTIVATION REACTIONS OF NEREIS EGGS

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The peculiar effects of 2,4,6-trinitrophenol (picric acid) in relation to the artificial activation of the eggs of *Nereis limbata* have been described in an earlier report (LeFevre, 1945). Discovery of these effects grew from a reinvestigation of a few experiments by Heilbrunn (1925) concerning the enhancing effect of acidification on the heat-activation of these eggs. Among a series of rather unrelated organic acids tested, only picric acid exerted a reliable activity of this sort, and further experimentation with this compound revealed a rather paradoxical set of properties with regard to the egg activation. Thus, at a concentration of about M/1000 in sea water, trinitrophenol prevented breakdown of the germinal vesicle, and accompanying cytoplasmic reorganization, ordinarily produced in these cells by exposure to heat (Just, 1915), excess potassium ion, or decalcifying agents (Wilbur, 1941). At the same time, exposure of the eggs to the same concentration of trinitrophenol rendered them subsequently hypersensitive to these same activating agents, so that upon removal from the acid they could be activated by exposure to these agents in doses too small to activate untreated eggs. This development of hypersensitivity was progressive with continued exposure to trinitrophenol, until after several hours the eggs were activated simply by removal to sea water, without application of any additional chemical or physical stimulant. The eggs remained in this state of hypersensitivity in the trinitrophenol for as long as three days, by which time in sea water they would long since have cytolyzed.

Various hypotheses were considered in an effort to integrate these diverse influences of trinitrophenol on the reactions of activation into a simple coherent pattern, in relation to some of the established factors relating to this process in the *Nereis* egg. The importance of the calcium ion in this connection (Heilbrunn and Wilbur, 1937; Wilbur, 1939, 1941) directed attention to the question of a calcium picrate complex formation, but there seemed to be no physicochemical basis for this; complications confronting interpretation of the effects of trinitrophenol in terms of a calcium-release theory are considered in the previous report (LeFevre, 1945). The least involved interpretation capable of explaining the behavior of the trinitrophenol seemed to be that this substance might form a reversible combination with some substance produced in the course of the cell's metabolic activities; that this substance could precipitate the breakdown of the germinal vesicle, but that it is normally removed by chemical reaction or by diffusion from the cell before activating concentrations are attained. This hypothesis would attribute the stimulating effects of the various agents to an increased rate of production of the activating metabolite, which can then take part in the critical reactions. Inhibition of this stimulation by trinitrophenol was attributed to formation of an inactive complex between the acid and the hypothetical activating substance. This interpretation accounted for the progressive development of hypersensitivity to activating agents during exposure to trinitrophenol, since removal from the acid bath to ordinary sea water, with accompanying rapid loss of picrate, would then release the accumulated activator, and would be equivalent to the application of an activating agent. The original report may be consulted for a more complete consideration of this hypothesis in the light of the earlier experiments. The present report is an extension of the above investigation in an attempt to elucidate the nature of the hypothetical reactions suggested by the original work.

Materials and Methods

Two main methods of approach have been employed: chemical disturbance of the egg-suspension medium, either during the sensitizing procedure with trinitrophenol or during application of chemical activating agents; and treatment of the eggs with substituted phenols other than picric acid, to ascertain the molecular specificity of the activities described for the latter.

The materials and general handling procedures have been previously described (LeFevre, 1945). In those experiments reported below involving treatment of egg-suspensions with gas mixtures, the appropriate quantities of carbon dioxide, unpurified commercial nitrogen, and air were drawn into a glass vessel of several liters capacity, by removal of water through a siphon; from this vessel the mixture was similarly forced in fine bubbles through 25–35 ml. of the egg-suspension in a 10–15 cm. column in an ordinary test tube. In addition to gas mixtures, specific metabolic inhibitors tested included potassium cyanide, sodium azide, sodium iodoacetate, hydroxylamine, *p*-chloromercuribenzoic acid, cupric chloride, urethane, and diethyl ether. Substituted phenols tested included 2,4,6-trinitrophenol, 2,4-dinitrophenol, *o*-nitrophenol, *p*-nitrophenol, 2,6-dichlor,4-nitrophenol, *o*-chlorophenol, and *p*-chlorophenol.

The artificial sea water medium used in some of the experiments was made up by mixing of several pure salt solutions isotonic with sea water: NaCl, 0.52 M, 500 volumes; KCl, 0.53 M, 10 volumes; $MgCl_2$, 0.37 M, 40 volumes; $MgSO_4$, 0.96 M, 15 volumes; $NaHCO_3$, 0.52 M, 2 volumes; $CaCl_2$, 0.34 M, 15 volumes. For those experiments involving upset of the normal proportions of the ingredients of sea water, corresponding changes were made in the relative volumes of the isotonic solutions mixed, so that the resultant surplus or deficit in total electrolyte content was taken up by all the other ingredients in their usual proportions.

Results

I. Experiments concerning reactions accompanying activation

a. Effects of anoxia

The dependence of the germinal vesicle breakdown response on aerobic processes was tested only in the case of activation by removal to sea water from prolonged exposures to trinitrophenol (10^{-3} M in sea water). There was no evidence of activation when the eggs were transferred to sea water through which a mixture of 95 per cent N_2, 5 per cent CO_2 had been passed for about thirty minutes, while 100 per cent activation was ordinarily obtained in undisturbed sea water or in sea water gassed with air or 95 per cent air, 5 per cent CO_2. This inhibition of activation in a nitrogen atmosphere was reversible for several minutes; Table I shows a typical instance of this, in which the eggs, upon removal from the anoxic bath to ordinary sea water, were activated in progressively diminishing numbers as the interval in the anoxic medium was extended.

b. Effects of metabolic inhibitors

The usual chain of events following removal of eggs from trinitrophenol baths to sea water was similarly inhibited by ether, potassium cyanide, or sodium azide; these inhibitors, however, did not prevent a slight elevation of the membrane, and the very first indications of the onset of nuclear reorganization, as previously described in eggs exposed to mixtures of sea water and isotonic KCl or sodium citrate in the presence of trinitrophenol (LeFevre, 1945). This initial disturbance was "frozen" at a very early stage, so that the germinal vesicles retained their identity, and none of the cytoplasmic rearrangements consequent to vesicular breakdown occurred. If, subsequently, the inhibitor was removed, even after several hours of such suspended activation, the usual processes continued normally; i.e., the inhibition was entirely reversible. In the case of ether, in fact, gradual evaporation of the narcotic led ultimately to a resurgence of the activation reactions, without actual removal of the eggs to fresh sea water. A typical set of experiments is presented in Table II. It may be noted that this reversibility outlasted that observed in anoxia by a wide margin, but this may have been attributable to the more rapid onset of irreversible injury to the eggs in the absence of oxygen. The critical concentrations for inhibition were those used in the experiments cited in Table II.

TABLE I

Reversible inhibition of activation by anoxia

Sea water under atmosphere of:	Per cent activation
Air	98
95% N_2, 5% CO_2	0
Removed, exposed to air	
after 1 min.	76
3 min.	60
5 min.	54
10 min.	32

Activating procedure: eggs removed to sea water, under atmosphere indicated, after exposure for three hours to trinitrophenol, M/1000, in sea water.

The same concentrations of ether, azide, or cyanide were equally effective in preventing activation in a few tests made with mixtures of sea water and isotonic KCl or sodium citrate (Table II); reversibility of this inhibition was not tested, but the high percentages of "incipient activation" observed would indicate the same state of affairs as seen with these inhibitors following the sensitization procedure. Hydroxylamine, at 10^{-2} M, also prevented activation by these agents, but the inhibition was irreversible, and although there was no immediate obvious morphological change, inhibition in this instance probably indicated nothing more than complete inactivation of the cells.

The other inhibitors tested in this same connection, rather than preventing activation, proved to be themselves activating agents. The same nuclear and cytoplasmic changes accompanying treatment with excess potassium, heat, etc., were seen when the eggs were exposed to sodium iodoacetate, at ca. $2 \cdot 10^{-2}$ M, or to urethane, at ca. $5 \cdot 10^{-3}$ M. At these concentrations, and throughout a fairly extensive range above these figures, 90–100 per cent germinal vesicle breakdown was regularly observed and was frequently followed by extensive irregular cleavage, though no swimming forms were found. Activation by these means resembled that induced by other agents in that it failed in the presence of trinitrophenol at 10^{-3} M. *p*-Chloromercuribenzoate or $CuCl_2$ (like iodoacetate, inhibitors of sulfhydryl activity) also activated the eggs, at concentrations in the neighborhod of 10^{-5} M; but this reaction was less easily reproducible than with the other stimulating "inhibitors," as the threshold concentration for activation was only slightly lower than the lytic concentrations, and there was some variability in the effective concentrations for different batches of eggs. A further difference in the activation in these instances is that that induced by Cu^{++} or *p*-chloromercuribenzoate was not interfered with by trinitrophenol. Thus activation by these agents appears not to be comparable to that otherwise induced, but more analogous to the prelytic activation seen in other instances (Loeb, 1913; Lillie, 1926).

TABLE II

Reversible inhibition of activation by metabolic inhibitors

Activating agent	Inhibitor	Per cent activation		
		Without inhibitor	With inhibitor	Inhibitor removed after 7 hours
Sea water, after 5 hours in trinitrophenol, M/1000, in sea water	Ether, 1%	100	0, 80*	100
	KCN, 10^{-3} M	100	0, 100	100
	NaN_3, 10^{-2} M	100	0, 50	100
KCl, 20 vol. isotonic, to 80 vol. sea water	Ether, 1%	100	0, 70	
	KCN, 10^{-3} M	100	0, 75	
	NaN_3, 10^{-2} M	100	0, 100	
Sodium citrate, 25 vol. isotonic, to 75 vol. sea water	NaN_3, 10^{-2} M	94	0, 90	

* The second figure in this column is the approximate percentage of eggs in the inhibitor showing the condition of "incipient activation" (elsewhere described).

c. Effects of ionic components of sea water

The regular response of the eggs upon removal to sea water after several hours' exposure to trinitrophenol, 10^{-3} M in sea water, raised the question of what components of sea water were essential to this response. If the eggs were removed from the conditioning bath not to sea water, but to isotonic solutions of either NaCl, KCl, $CaCl_2$, or $MgCl_2$, no stimulatory reaction was ever observed. Mixtures of various proportions of these basic ingredients were then tested. In the absence of magnesium, there was always such a distortion of the egg contours, with extreme membrane elevation and discoloring, that it was difficult to analyze other differences that might appear. It was however evident that the addition of calcium, in the absence of magnesium, greatly augmented these disruptive changes, leading to decided cellular deformation and nuclear disarrangement resembling in some respects the changes that occur in activation, and frequently to extensive cleavage, but without any change in the appearance of the cytoplasm. The deleterious effects were further exaggerated in the presence of bicarbonate buffer, with a characteristic red-brown discoloration and vesiculation of the protoplasm. In an isotonic solution containing $MgCl_2$, NaCl, and $NaHCO_3$, with the Na^+, Mg^{++}, and HCO_3^- in proportions similar to those found in sea water, the eggs remained perfectly normal in appearance. But this mixture was not capable of inducing the reactions of activation in eggs transferred from a conditioning bath with trinitrophenol. Activation required the presence of at least a reasonable trace of calcium ion; and at a given calcium ion level the reaction was augmented by increased potassium ion concentration, as in the typical experiment shown in Table III. If both calcium and potassium were present at M/100, the reaction was as easily elicited as in sea water. To some extent, increased concentration of magnesium ion antagonized this stimulatory activity, so that more calcium and potassium were required to elicit the same percentage of response. The specific necessity for calcium was verified by varying its concentration in the otherwise fairly complete artificial sea water medium described in an earlier section; reduction of the calcium level to half the normal figure produced a noticeable diminution in the percentage of response, although there was still some degree of response even with only one-tenth of the normal calcium concentration.

TABLE III

Action of calcium and potassium ions in initiation of activation

Concentration of potassium ion	Per cent activation with calcium ion concentration of:			
	0*	0.01 M	0.02 M	0.04 M
0*	0	0	50	66
0.005 M	0	93	92	98
0.01 M	0	100	100	100
0.02 M	3	100	100	100

* On basis of absolute freedom of reagents from Ca^{++}, K^+-contamination, and ignoring small amount carried over in sea water with eggs. Medium was made up of NaCl, 0.47 M; $MgCl_2$, 0.037 M; $NaHCO_3$, 0.0026 M; plus $CaCl_2$, KCl as indicated for each case. Eggs removed to medium indicated after exposure for 7 hours to trinitrophenol, M/1000, in sea water.

The necessity of calcium, in initiating the stimulatory changes upon removal from exposure to trinitrophenol, was immediate, as shown in Table IV. Addition of calcium to the system only 60 seconds after the removal of the trinitrophenol did not result in a significant amount of activation. Thus in this sense the inhibition of activation by calcium-lack may be said to be irreversible. This effect is not, however, attributable to general injury to the cells because of the lack of calcium, as the same cells may subsequently be activated by any of the usual procedures. Also, the eggs remained fertilizable for as long as eight hours in artificial sea water as nearly Ca-free as the purity of the reagents permitted. Such eggs, however, always lost their fertilizability and cytolyzed some time before those in the control medium.[1]

TABLE IV

Immediate necessity of calcium in activation following sensitization

Condition	Per cent activation
In artificial sea water	50
In Ca-free artificial sea water	0
Removed, to complete artificial sea water, after 15 sec.	51
30 sec.	36
60 sec.	10
90 sec.	0
120 sec.	0
180 sec.	0

Eggs removed to medium indicated after exposure for 24 hours to trinitrophenol, M/1000, in sea water.

In a similar manner, depletion of the calcium content of the medium prevented activation of the eggs by Wilbur's methods, addition of isotonic KCl or sodium citrate to the sea water medium. Citrate activation was especially sensitive to low calcium concentration, failing entirely if as little as ⅔ of the calcium was removed; this was particularly curious, since the citrate in itself would be expected to render unavailable most of the calcium in the medium.

II. Experiments concerning reactions accompanying sensitization

a. Effects of anoxia

Comparisons were made of the rates of development of sensitivity to sea water, during exposure to trinitrophenol, in the presence of various gas mixtures. Gassing the trinitrophenol solution (10^{-3} M in sea water) with nitrogen, or with 95 per cent nitrogen, 5 per cent carbon dioxide, led to a decidedly earlier development of sensitivity (Table V); but with more prolonged exposures, the eggs in the anoxic baths became irreversibly inactivated, and began to cytolyze, long before any evidence of damage appeared in the control aerated dishes. Increased percentage of CO_2 in mixtures with air, at least up to 50 per cent CO_2, similarly enhanced the development of sensitization, and did not appear to injure the eggs subsequently. It was frequently observed in other experiments that the packing of eggs in considerable thicknesses at the bottom of the container, during exposures to trinitrophenol, led to more rapid development of sensitivity than appeared when more dispersed, thinner layers of eggs were used. It seems likely that this effect is to be attributed

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[1] On the other hand, less pronounced depletion of the calcium, down to about 1/100 the normal level, progressively delayed the onset of cytolysis; this effect may be due simply to the calcium requirements of micro-organisms responsible for the disintegration of the eggs, but Schechter (1937a, b) has described similar effects of Ca^{++}-reduction in a number of species of commonly used marine eggs.

TABLE V

Enhancement by anoxia of sensitization in trinitrophenol

Duration of sensitizing bath min.	Per cent activation following sensitization under atmosphere of: 95% N_2 5% CO_2	95% air 5% CO_2
4	0	0
16	7	1
41	100	6
59	100	63

Eggs removed to fresh sea water after indicated exposure to trinitrophenol (10^{-3} M in sea water) under atmosphere described.

to the higher CO_2 or the lower O_2 tension (or both) in the immediate environment of the cells packed in greater density.

b. Effects of metabolic inhibitors

Short of blocking subsequent activity by killing the cells (as indicated by loss of fertilizability, rapidly followed in most instances by disintegration), no reliable influence on the rate of sensitization in trinitrophenol was observed in the presence of ether, urethane, or sodium iodoacetate. (See section *d* below.) Hydroxylamine, at 10^{-2} M, or potassium cyanide, at ca. 10^{-3} M,[2] markedly enhanced the rate of sensitization, the more so as the concentration was increased; a less reliable similar activity was seen with sodium azide, in the neighborhood of $5 \cdot 10^{-3}$ M. Table VI shows a typical set of results with various concentrations of KCN. In all of these cases, the stimulatory action was superseded by lethal effects, more readily at the higher concentrations, the maximum stimulating action then passing progressively to the lower concentrations, as in the instance presented in Table VI. This cytolysis occurred following the transfer to sea water, not in the trinitrophenol baths containing the inhibitor; this is as would be expected if a great excess of the activator led to cytolysis rather than activation, as was also suggested in the older experiments.

TABLE VI

Enhancement by cyanide of sensitization in trinitrophenol

Duration of sensitizing bath min.	Per cent activation when sensitized with KCN at concentration of: $5 \cdot 10^{-3}$ M	10^{-3} M	$2 \cdot 10^{-4}$ M.	0
72	100	27	39	4
127	52	68	61	2
432	0	97	75	62
1554	Cytolyzed	Cytolyzed	100	99

Eggs removed to fresh sea water after indicated exposure to trinitrophenol (10^{-3} M in sea water) with added KCN as shown.

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[2] Cyanide and trinitrophenol reacted slowly in these preparations, progressively developing an amber color of unknown significance, over a period of several hours. With higher concentrations of cyanide, the rate of development of this discoloration was correspondingly higher.

c. *Effects of ionic components of sea water*

Following the experiments described above, in which it appeared that the calcium ion was the specifically essential component of sea water in permitting the initiation of activation, possible involvement of this ion in the reactions of sensitization was tested. Removal of calcum ion from the artificial sea water medium, containing trinitrophenol at M/1000, produced no evident change in the rate of sensitization; this absence of effect was noted with calcium contents as low as 1/60 the normal level. More complete removal was impracticable, since in such low-Ca^{++} media the trinitrophenol became rapidly toxic to the cells and led to cytolysis before the sensitization had gotten well under way. This protective effect of calcium against destruction of the eggs by the acid was evident in comparison of the normal medium with that containing as much as 1/10 the normal amount of calcium ion.

Since it was evident from experiments considered above that increased CO_2 tension hastened sensitization in trinitrophenol, it seemed expedient to test the effects of alterations in the related buffer system. But simple neutralization of the trinitrophenol (readjusting the pH from 6.7–7.0 back to 8.0 as in sea water) had no demonstrable effect on the rate of sensitization. In nearly all of the experiments with trinitrophenol, this neutralization was routinely performed. In a single experiment, the amount of bicarbonate in equilibrium with atmospheric CO_2 was varied in artificial sea water media, from zero to three times the normal amount, and eggs were exposed to neutralized trinitrophenol, 10^{-3} M, in each of these mixtures. Throughout this range, the sensitization rate was more rapid, the more bicarbonate present (Table VII). The effect of CO_2 is thus probably not to be attributed to its acidifying effects in the medium, but to its action after passing intracellularly, as in the effects described by Jacobs (1920).

TABLE VII

Effect of bicarbonate on sensitization in trinitrophenol

Duration of sensitizing bath min.	Per cent activation following sensitization in trinitrophenol, 10^{-3} M, in artificial sea water with bicarbonate content of:			
	3 × usual	Usual (0.0018 M)	0.3 × usual	None
25	0	0	0	0
92	0	0	0	0
156	2	0	0	0
212	38	4	3	1
405	100	77	3	0
1307	100	100	100	100

d. *Effects of stimulating agents*

One deduction from the hypothesis of the activator-substance (LeFevre, 1945), subject to experimental test, is that the activator should accumulate more rapidly if the eggs in the trinitrophenol bath are simultaneously exposed to an agent which would bring about activation in the absence of the inhibitor. As previously reported, this proved to be the case in only about ⅕ of the tests when heat was used as the activating agent, the remaining ⅘ of the results showing no significant differences in either direction.

The same procedure applied to mixtures of sea water and isotonic KCl or sodium citrate gave different results with the two agents. With addition of extra potassium to the sensitizing picrate baths, there was invariably a more rapid development of the capacity to react upon removal to sea water, similar to that shown with other agents in Tables V, VI, and VII. This was in accord with the predictions of the hypothesis; in no case, however, did such a difference appear in the presence of the citrate stimulant. Also, as noted above, the stimulants urethane and iodoacetate did not regularly enhance the development of sensitivity in trinitrophenol; each of these substances exerted this effect in a few instances, and in no case produced a slowing of the sensitization, so that their position in this regard is the same as that of the heat stimulus originally investigated.

III. *Experiments concerning the effectiveness of substituted phenols other than picric acid*

a. *Blocking of activation*

Several substituted phenols differing from trinitrophenol in the nature or position of the substituted groups on the phenol were compared, all at 10^{-3} M, the concentration at which standard experimentation with trinitrophenol was carried out.[3] 2,4-Dinitrophenol and *p*-nitrophenol were more effective than trinitrophenol in blocking activation by either KCl-sea water mixtures or sodium citrate-sea water mixtures; in one case only, *p*-nitrophenol allowed a small number of eggs to reach the stage previously designated as "incipient activation." *o*-Nitrophenol was almost as effective against citrate activation, and was superior in this respect to trinitrophenol; in spite of this fact, *o*-nitrophenol had absolutely no influence on activation by excess potassium. 2,6-Dichlor,4-nitrophenol was approximately equivalent to 2,4,6-trinitrophenol in its inhibitory action, usually allowing a moderate percentage of "incipients." *o*-Chlorophenol and *p*-chlorophenol usually prevented activation, but these effects appeared to be attributable to irreversible damage to the eggs. Prolonged exposure to the chlorophenols led to cytolysis, the initial stages of which, as noted by many investigators in artificial parthenogenesis, resemble in some respects the early stages of activation, so that it at times appeared upon cursory examination that these chlorophenols augmented the stimulating action of the chemical agents used. Phenol itself had no inhibitory effect whatsoever at this concentration.

In summary, the inhibitory effect of picric acid was duplicated by each tested phenol which had a nitro-group in the para-position; the only comparable action seen in the absence of this group was that of *o*-nitrophenol with respect to citrate activation.

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[3] If neutralized, trinitrophenol at concentrations as high as 10^{-2} M were tolerated for many hours, and were very effective in blocking and sensitizing, but the concentration of 10^{-3} M gave the most prolonged effects without cell damage. Increasing the concentration past 10^{-3} M did not increase the rate of development of sensitivity to sea water in eggs exposed to the trinitrophenol; this independence of the rate of sensitization from the trinitrophenol concentration is in harmony with the hypothesis advanced in the discussion concerning the mode of action of the trinitrophenol.

b. Sensitization to sea water

At 10^{-3} M, *o*-nitrophenol, 2,4-dinitrophenol, *o*-chlorophenol, or *p*-chlorophenol had no sensitizing effect on the eggs in exposures up to 24 hours or until the onset of cytolysis. Cytolysis occurred within a few hours in *p*-chlorophenol, after about 20 hours in *o*-chlorophenol or dinitrophenol, but no sooner in *o*-nitrophenol than in sea water. *p*-Nitrophenol showed some sensitizing activity, as indicated in Figure 1, but only after about 10 hours, and this effect disappeared rather rapidly, so that cytolysis began to be evident at about 20 hours. The dichloronitrophenol sensitized the eggs to sea water as rapidly as, or perhaps a little more rapidly than, trinitrophenol, but, like *p*-nitrophenol, led to cytolysis within about 20 hours (Fig. 1).

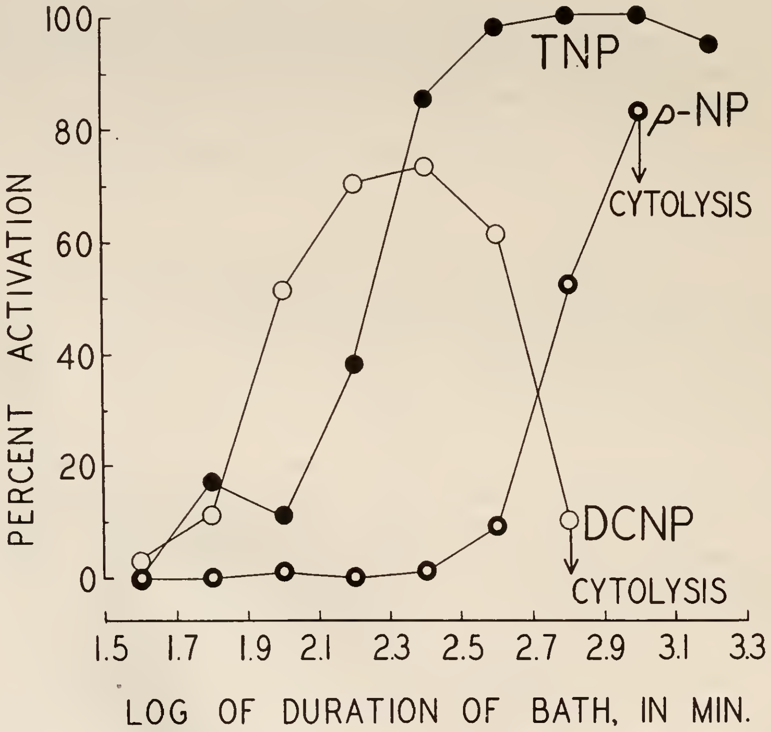

FIGURE 1. Comparison of sensitization rates in the three effective substituted phenols found.

Eggs removed to sea water following bath in either 2,4,6-trinitrophenol (TNP), *p*-nitrophenol (*p*-NP), or 2,6-dichlor,4-nitrophenol (DCNP); all at 10^{-3} M, in sea water.

Each point is the average of all experiments (usually three or four) performed in the logarithmic time interval marked at the base-line. Each individual experiment compares the action of the three phenols on the same batch of eggs.

Thus, it seemed apparent that again the $-NO_2$ group in the para-position on the phenol was the primary essential, the groups in the ortho-positions exerting only modifying influences on the sensitizing reactions. However, unlike the situation observed in the study of inhibition of activation, there was no sensitizing activity evident with 2,4-dinitrophenol, which contains the apparently critical group. This is especially odd in view of the fact that either the addition of another $-NO_2$ in the 6-position, or removal of the $-NO_2$ from the 2-position, produces a highly active substance (many swimming "larvae" were obtained from the use of trinitrophenol or *p*-nitrophenol). Also, substitution of $-Cl$ for both of the $-NO_2$ groups in the ortho-positions did not interfere wth the sensitization activity.

However, of all these, only 2,4,6-trinitrophenol (picric acid) was effective in the lengthy preservation of the eggs against death and cytolysis; the others were all, in fact, somewhat toxic, leading to cytolysis within the first day of exposure. This may mean that the two nitro-groups in the ortho-positions serve to detoxify the molecule, perhaps preventing other reactions not basically related to those involved in the reversible inhibition and sensitization under study.

Discussion

None of the newly acquired data is antagonistic to the hypothesis developed from the results previously reported, involving the concept of the metabolite activator-principle. However, no crucial experimental test of this hypothesis has as yet been conceived; the present data concern the general nature of the reactions accompanying chemical activation and sensitization of the eggs, insofar as this is revealed by aberrations in these reactions with changes in the chemical environment.

Thus it is apparent that some phase of the reactions accompanying the process of germinal vesicle breakdown involves an oxidation employing molecular oxygen, probably through a cytochrome system, since this process was reversibly prevented by anoxia, cyanide, or azide. However, as noted by Barron (1932), in the activation of *Nereis* eggs by actual fertilization by sperm in very complete anoxia, this inhibition affects not the initiation of activation, but the later nuclear changes and consequent development. Diethyl ether also prevented these reactions, but there is less specificity in its action, so that interpretation of this inhibition is more indefinite.

With the exception of ether, the same inhibitors which prevented activation also hastened the onset of sensitization of the eggs exposed to trinitrophenol, so that the cells were activated, upon removal from the acid to plain sea water, following a shorter stay than required in the absence of the inhibitors. This fact is readily incorporated into the general hypothesis, since inhibition of the activating reactions would be expected to lead to more rapid accumulation of the activator-trinitrophenol complex, by eliminating one of the routes by which the free activator might be otherwise removed. Interpretation might also be sought on the basis of the acidifying effects of these inhibitors intracellularly, with consequent release of Ca^{++} from combination with cellular proteins. The fact that the inhibitors never appeared to induce sensitization in themselves, together with the temporal characteristics of the sensitization, would necessitate a rather unwieldy complex of assumptions about the behavior of the Ca^{++} and trinitrophenol, in application of these interpretations (LeFevre, 1945).

The observed results would however be expected if there were any alternative pathway by which the activator could be removed, either by chemical reaction or by diffusion from the cell; or simply if the reaction in which the activator is released were reversible and governed by mass action. In the latter case, accumulation of the activator in the presence of these metabolic poisons would be self-limited, whereas large amounts could be accumulated in inactive form with trinitrophenol, and this process would be accelerated by addition of the inhibitors; this would account for all the relevant experimental results with a minimum of independent hypotheses.

As noted above, the metabolic inhibitors which prevented activation exert their influence not on the immediate activating disturbance (visible at the egg surface) but on the immediately subsequent cellular reorganization. On the other hand, inhibition of the same over-all process ("activation") by removal of calcium ions from the medium acted at the very earliest stages in the chain of events. The absence of calcium ion at the critical instant of potential initiation of activation was thus an irreversible disturbance, and the cell did not react upon replacement of the missing element unless a second stimulus was applied. Heilbrunn (1925), Heilbrunn and Wilbur (1937), and Wilbur (1939, 1941) concluded from experiments along various lines that the reaction in question is dependent on rearrangement of the intracellular calcium with respect to the protoplasmic colloids, with accompanying changes in viscosity. The modifying influences of potassium and magnesium ions, as reported above, are in keeping with the general pattern of these cations in affecting colloidal reactions with calcium ion, as described by Heilbrunn in numerous cellular reactions.

Other aspects of the effects of metabolic poisons on the reactions of activation are not so readily interpreted; it is particularly odd, though not entirely without parallel, that germinal vesicle breakdown is initiated by exposure to iodoacetate or urethane (or, in a different manner, by application of Cu^{++} or *p*-chloromercuribenzoate), which substances are generally recognized as inhibitors or narcotics. The most evident interpretation of these results in the light of the related observations is that these inhibitory agents may prevent some alternative reactions of the activator substance or its precursors, so that the activator concentration is increased by the presence of the inhibitors. Obviously no specific characterization of the hypothetical reactions involved can be made, except that it seems likely that some enzyme concerned contains active sulfhydryl groups.

Some special mention should be made of the fact that there is not complete agreement in the results obtained with the various procedures employed. Some of these discrepancies are easily dismissed as quantitative differences in effects of the activators and inhibitors on the critical reaction rates. However, the distinct inhibition of citrate activation by *o*-nitrophenol is entirely out of line with all other relevant data; citrate in stimulatory concentrations also invariably failed to hasten sensitization of the eggs in trinitrophenol.

Beyond the considerations outlined, the present data do not permit identification of the hypothetical substances or their reactions; perhaps some clue is afforded in the apparent specificity of the *p*-nitrophenol grouping in the reversible formation of an inactive complex with the activator. The author is not prepared to interpret this finding; innovation in experimental approach is probably necessary before a more coherent pattern will emerge from the diverse observations reported in this paper and in the earlier report. The interpretations offered seem the least involved and most comprehensive of the facts available at this time.

SUMMARY

1. Inhibition of activation of *Nereis* eggs by trinitrophenol, with concurrent sensitization of the eggs to subsequent stimulation, appears to depend on the nitro-group in the para-position on the phenol.

2. The rate of the sensitization process is enhanced by anoxia, CO_2, inhibitors of the cytochrome system, or increased potassium ion concentration, but is insensitive to several other inhibitors, narcotics, stimulating agents, and to calcium ion deprivation.

3. The immediate initiation of activation by various chemical procedures requires the presence of the calcium ion, is assisted by the potassium ion, and slightly depressed by increasing magnesium ion concentration, but is not affected by anoxia or by various metabolic poisons.

4. Subsequent nuclear and cytoplasmic reorganization, ensuing some minutes after the initial disturbance, is reversibly inhibited by anoxia, inhibitors of the cytochrome system, or diethyl ether.

5. Urethane and iodoacetate activate the eggs; this activation is inhibited by trinitrophenol. Cupric ion and *p*-chloromercuribenzoate also activate the eggs, but only at nearly lytic concentrations, and the activation is not affected by trinitrophenol.

6. These data are partly interpreted in relation to the hypothesis of an activator metabolite produced within the egg.

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THE ACTION OF CHOLINE AND RELATED COMPOUNDS ON THE HEART OF VENUS MERCENARIA[1]

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Since the demonstration by Prosser (1940) of the unusual sensitivity to acetylcholine (Ach) of the isolated heart of the bivalve molusc, *Venus mercenaria,* we have extensively employed this preparation for the bio-assay of Ach in tissue extracts (e.g. Welsh, 1943; Welsh and Hyde, 1944a and b; Prajmovsky and Welsh, 1948). In certain respects it is superior to the classical Ach assay preparations such as the dorsal muscle of the leech, rectus abdominis of the frog, isolated frog heart, and blood pressure of cat. For example, it is more sensitive to Ach, with complete inhibition occurring at about 50 times the threshold inhibitory concentration; it is relatively unaffected by changes in pH, inorganic ions, and tissue constituents other than Ach; it recovers quickly, thereby allowing more rapid estimation than the above-mentioned preparations.

While employing the *Venus* heart for bio-assay, its responses to a variety of drugs, organic compounds, and inorganic ions have been studied and, in particular, to a series of choline esters and analogs—this in the hope of obtaining evidence toward a better understanding of the fundamental mode of action of Ach. An organ with a high specificity for choline esters, exhibiting a response which is easily quantified, and which has so little self-contained cholinesterase that blocking of this enzyme is not necessary when working at great dilutions of the unstable esters, provides a suitable object for studying certain aspects of the mechanism by which Ach acts on cells.

The present paper has two purposes: (1) to indicate the methods of preparing and employing the *Venus* heart for the bio-assay of Ach, and (2) to compare the effects of other choline esters and related compounds which differ in greater or less degree from the Ach molecule.

METHODS OF PREPARING AND USING THE HEARTS FOR ACH ESTIMATION

An earlier paper by Wait (1943) covers some of the necessary procedures for preparing the isolated *Venus* heart for Ach determinations. For convenience, however, the steps which we employ from the securing of appropriate test animals to the quantitative estimation of Ach in tissue extracts will be outlined.

Venus mercenaria (the hard shell clam or quahog), being an important commercial shellfish along the Atlantic Coast, are usually available where shellfish are sold. They remain edible for some weeks after digging or dredging, if maintained under refrigeration, but after one to two weeks the hearts of such animals tend to beat with an irregular rhythm; it is important, therefore, to obtain experimental

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material from a source where the previous history is known [2] and to use this material within one to two weeks after removal from the sea. *Venus* with a shell length of 8 to 12 cm. have been found of most convenient size. In the laboratory they may be stored dry at 5°–10° C., or preferably kept in shallow tanks of aerated sea water, at 15°–18° C.

The heart is exposed by breaking and removing the dorsal portion of the shells (umbos and hinge) and then cutting away the mantle and pericardium dorsal to the heart. The heart consists of a single, median ventricle with laterally disposed, thin-walled atria (auricles). Anterior and posterior blood vessels leave the heart in close association with the intestine which passes through the heart. Threads for attaching to a support in the bath and to the writing lever may be passed under the atria and tied close to the ventricle in order to include some of the thicker-walled, ventricular muscle. Cutting the atria distal to the threads, and cutting the blood vessels and intestine, isolates the ventricle which may then be placed in an appropriate heart bath. Only the outer surface of the heart is directly exposed to materials introduced into the bath, but cannulation of the heart and introduction of Ach into the ventricle does not increase its sensitivity.

The bath figured by Wait (1943) is satisfactory unless temperature control is desired (e.g. when working in a room above 20° C., or when maximum sensitivity is required); then a bath with a water jacket through which water of an appropriate temperature (15°–18° C.) is circulated may be employed, or the heart bath may be placed in a larger temperature-controlled vessel. It is necessary that provision be made for changing the fluids of the heart bath without draining the bath and subjecting the heart to undue mechanical disturbance. A bath holding 10 ml. when filled has been found appropriate.

An analysis of the inorganic salts of the blood of *Venus mercenaria* by Cole (1940) showed only small differences in comparison with sea water. It is not surprising, therefore, that sea water is an adequate perfusion fluid for the isolated heart, allowing a regular beat to be maintained for 2–3 days. Where natural sea water is not available, an artificial perfusion fluid may be used and several have been tried, with differing ratios of the common ions, without noting any appreciable effects on the heart until radical departures from the normal concentrations of the common ions are made. A fluid found satisfactory has the following composition: 30 gm. NaCl; 0.9 gm. KCl; 1.1 gm. $CaCl_2$; 3.5 gm. $MgSO_4 \cdot 3H_2O$ in one liter of water with a phosphate or bicarbonate buffer (pH 7–7.5). Changes of pH between 6 and 8.5 have little or no effect on the amplitude or frequency of beat, or on the response of the heart to acetylcholine for periods of time up to several hours.

Oxygen may be supplied by air or a mixture of 95 per cent O_2–5 per cent CO_2 passed through the bath. The bubbles should be small to avoid mechanical disturbance to the heart. The gas mixture or air may be admitted to the bath through the hooked support for the lower attachment of the heart if this is made from glass tubing drawn out to a fine tip. The heart lever should be counterweighted to give a pull of 200–300 mg. A kymograph speed of about 2 cm. per minute is desirable. Substances to be tested may conveniently be added at the bottom of the bath by

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[2] E.g. Supply Department, Marine Biological Laboratory, Woods Hole, Mass., or a wholesale dealer in shellfish.

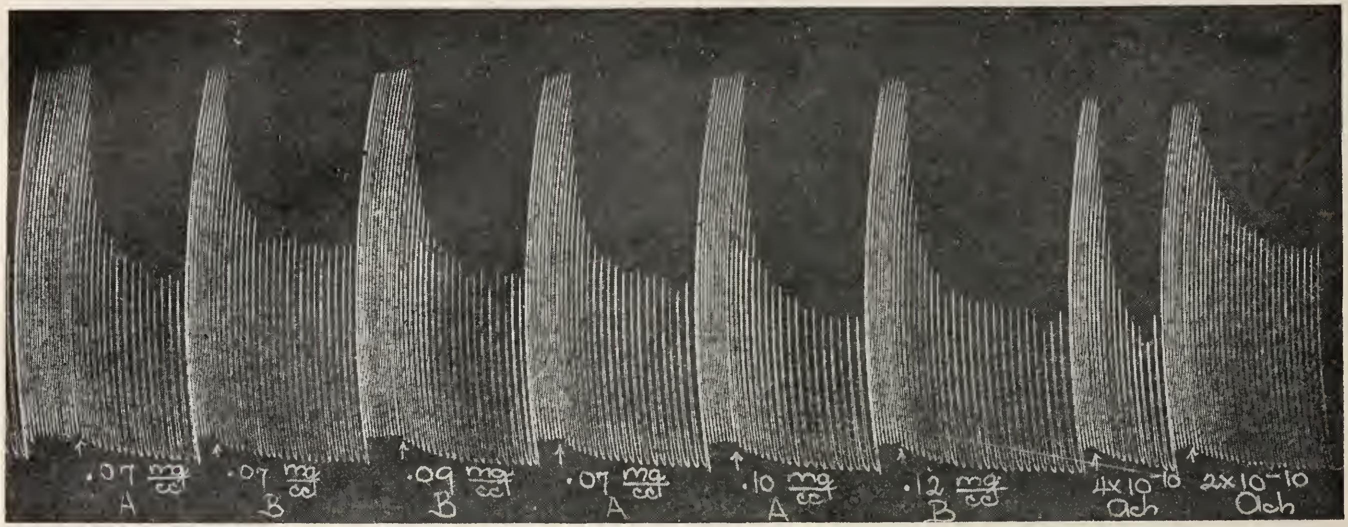


FIGURE 1. Sample record of bio-assay using the isolated *Venus* heart to estimate the amounts of extractable acetylcholine in two isolated ventral nerve cords (A and B) of nymphs of the cockroach, *Blaberus*. Total wet weights of tissue available: cord A = 17.3 mg.; cord B = 15.4 mg. The sensitivity of this heart to Ach and the approximate activities of the extracts had been determined in an earlier portion of the record. At the first arrow an amount of extract of cord A was added to the bath to give a final dilution of substance per cc. yielded by an original wet weight of tissue of 0.07 mg. The extract remained in the bath approximately one minute; the drum was stopped and the fluid of the bath changed several times and the heart allowed 3–5 min. to recover its original amplitude. After recording a few normal beats the extract of cord B was added at the second arrow. Following two attempts to determine amounts of extracts of cords A and B required to produce equivalent decreases in amplitude of beat, two known concentrations of Ach were applied (4×10^{-10} and 2×10^{-10} gm./cc.). From the results it was possible to calculate that cord A contained an Ach-like material equivalent to 4.0 μg. Ach/gm. wet weight while cord B yielded a value of 3.3 μg. Ach/gm. On a second heart the values obtained were A = 5.0 μg. Ach/gm.; B = 4.2 μg. Ach/gm.

means of a long hypodermic needle bent at a right angle; the volumes added should be small (1 ml. or less).

In estimating the Ach content of a tissue extract, a dilution should be found that gives between 20 and 80 per cent decrease in amplitude of beat at the end of one or two minutes. This should be matched, preferably twice, with known concentrations of Ach, after which appropriate calculations will give the Ach equivalent per gram of tissue. A sample record is shown in Figure 1. If it is suspected that substances in the tissue extract other than Ach are affecting the heart, it may be desirable to treat a portion of the extract by the addition of NaOH and warming in order to destroy the Ach present, and after neutralizing with HCl, to use this to make up the last dilution of Ach prior to adding to the bath.

Treatment of a heart with an anti-cholinesterase (physostigmine, neostigmine or di-isopropyl fluorophosphate) may potentiate the action of Ach two to five times. This small degree of potentiation is undoubtedly due to the low level of cholinesterase activity in these hearts (Smith and Glick, 1939; Jullien et al., 1938). Because the untreated heart is so sensitive to Ach (threshold for inhibition is usually between 10^{-10} and 10^{-11} gm. per ml.), and because recovery after Ach is slowed by treatment with an anti-cholinesterase, it is normally undesirable to employ this means for increasing sensitivity.

Occasionally hearts are encountered which fail to beat or which beat with a low amplitude. Although adrenalin and tyramine have been found to be excitants in relatively high concentrations (5×10^{-5} to 10^{-4} M) their effects are quickly abolished by washing. On the other hand ergotoxine, ergotamine, and ergonovine have been found to have a remarkably persistent excitatory action. For example, one part per million of ergotoxine ethanesulfonate will frequently cause renewal of heart beat, or an increase in amplitude of two to three times, in a heart with an abnormally low amplitude. Treatment for a few minutes with one of these ergot alkaloids produces a change in the physiology of the heart which persists for many hours in spite of repeated washings, while the response to Ach is affected but slightly. In this connection it should be noted that the *Venus* heart is composed of smooth muscle and its pharmacology is not unlike that of certain types of vertebrate smooth muscle.

There is some seasonal variation in the sensitivity of *Venus* hearts to Ach, with maximum sensitivity in the late winter and spring months (cf. Prosser, 1940; Wait, 1943), but the change is probably not as great as indicated by Prosser. More important to note is that in late summer there is a tendency toward irregularity in beat. This is often so pronounced that accurate estimates of small differences in Ach levels cannot readily be made in August and early September in the region of Massachusetts.

The Action of Certain Choline Derivatives and Related Compounds on the Venus Heart

The greatest gap in our knowledge concerning Ach is the precise manner in which this physiologically active substance affects the excitability of cells. More detailed studies of the mechanism of action of Ach are needed, and it would appear to matter little what type of Ach-sensitive tissue or organ is used in these studies. For many reasons the isolated heart of the quahog appears to be peculiarly suitable for such a study, and in this section of the present paper it will be shown that no compound related to Ach has yet been found having as great an inhibitory action on this organ as does Ach. It will also be shown that the methyl grouping around the onium element is, in many respects, the most significant portion of the Ach molecule.

The typical effects of Ach on the *Venus* heart will first be described, and then the relative activities of a number of common choline derivatives and certain related compounds will be discussed.

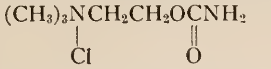
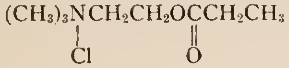
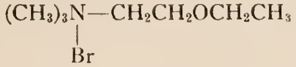
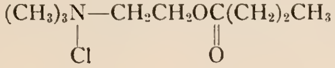
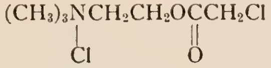
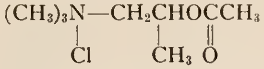
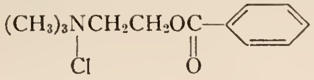
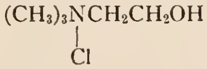
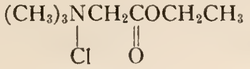
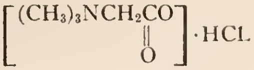
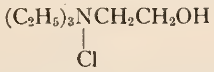
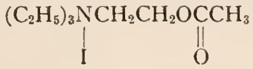
When Ach is added to a bath containing a beating *Venus* heart, which has received no previous drug treatment, to give a concentration in the vicinity of 10^{-11} to 10^{-10} M, a small increase in amplitude is sometimes observed. A similar stimulating action of low concentrations of Ach on vertebrate hearts has been observed by McDowall (1946) and others. At concentrations of Ach in the vicinity of 10^{-9} to 10^{-8} M a negative inotropic effect is seen; and with increasing amounts of Ach the amplitude of beat decreases until the heart stops in diastole at a concentration of Ach about 50 times that which gives a just measurable decrease in amplitude. Thus the range of concentrations from the threshold of inhibition to complete inhibition is relatively narrow. The log-concentration-response curve is sigmoid, with the portion between 20 and 80 per cent inhibition approximating a straight line. The inhibitory action of Ach on the *Venus* heart is more prominent and consistent than the excitatory action of lower concentrations, but it seems probable, as pointed out elsewhere (Welsh, 1948), that Ach first excites and then in higher concentrations inhibits or paralyzes this organ as it may do to all tissues or organs which respond to Ach.

In Table I a summary is given of the relative inhibitory activities of a number of compounds related to choline or Ach. The data shown in this table were obtained by finding a molar concentration of Ach that would produce between 20 and 80 per cent decrease in amplitude of beat of a given heart, and then the molar quantity of a related compound that would produce a degree of inhibition exactly matching that produced by the Ach. Each value shown for a given compound was obtained on a different heart. The average values may be taken as a fairly precise indication of the relative inhibitory effectiveness of these several compounds on the *Venus* heart. Since cholinesterase activity in this organ is extremely low, anti-cholinesterases were not employed, but the complications which may arise when stable choline esters are compared with unstable in the presence of active cholinesterases are believed to be minimal.

In commenting on certain of the more interesting facts given in Table I attention may first be called to choline. Choline affects the isolated *Venus* heart in a manner very much like that of Ach, except that it is far less active. When first applied in low concentrations, the amplitude of beat may increase (Fig. 2, Curve 1). Greater variation in the response of different hearts to choline was observed than in the case of any other compound. This is illustrated by Figure 2, where concentration response curves for three different hearts are shown. The wide range of values obtained when choline was compared with Ach may be accounted for by individual variation in the response to choline, for it is obvious that if a match of molar concentrations of Ach and choline producing 25 per cent inhibition were made on the heart represented by curve 3, the relative value for choline might be 1000; while a match of 25 per cent inhibition made on the heart represented by curve 2 would yield a value showing Ach to be perhaps 50,000 times as active as choline.

TABLE I

Relative molar quantities required to produce a decrease in amplitude of beat equivalent to that produced by a molar quantity of acetylcholine chloride equal to 1

Choline derivative	Structural formula	Molecular weight	Values obtained	Averages
Carbamylcholine chloride	 $(CH_3)_3\underset{Cl}{\underset{\mid}{N}}CH_2CH_2O\underset{O}{\underset{\|}{C}}NH_2$	182.5	20 20 25 40 40 50 80 100 100 150 250	80
n-Propionylcholine chloride	 $(CH_3)_3\underset{Cl}{\underset{\mid}{N}}CH_2CH_2O\underset{O}{\underset{\|}{C}}CH_2CH_3$	195	100 100 100 120	105
Ethoxycholine bromide	 $(CH_3)_3\underset{Br}{\underset{\mid}{N}}-CH_2CH_2OCH_2CH_3$	212	100 100 100 130 200 200 200 250	160
Butyrylcholine chloride	 $(CH_3)_3\underset{Cl}{\underset{\mid}{N}}-CH_2CH_2O\underset{O}{\underset{\|}{C}}(CH_2)_2CH_3$	209	200 500 800 1,000	625
Chloracetylcholine chloride	 $(CH_3)_3\underset{Cl}{\underset{\mid}{N}}CH_2CH_2O\underset{O}{\underset{\|}{C}}CH_2Cl$	215.9	500 750 1,000 1,600	960
Acetyl β methyl choline chloride	 $(CH_3)_3\underset{Cl}{\underset{\mid}{N}}-CH_2\underset{CH_3}{\underset{\mid}{C}}HO\underset{O}{\underset{\|}{C}}CH_3$	195	400 1,000 1,000 2,000	1,100
Benzoylcholine chloride		243	10,000 10,000 12,000 13,000 20,000	15,000
Choline chloride	 $(CH_3)_3\underset{Cl}{\underset{\mid}{N}}CH_2CH_2OH$	139.5	400 1,000 1,000 1,000 1,000 2,000 4,000 5,000 7,000 15,000 40,000 100,000	14,000
Betaine ethyl ester chloride	 $(CH_3)_3\underset{Cl}{\underset{\mid}{N}}CH_2\underset{O}{\underset{\|}{C}}OCH_2CH_3$	181.5	1,000 1,000	1,000
Betaine hydrochloride	 $\left[(CH_3)_3NCH_2\underset{O}{\underset{\|}{C}}O\right]\cdot HCL$	117	3,300 5,000 10,000 20,000 25,000 25,000	15,000
Triethylcholine chloride	 $(C_2H_5)_3\underset{Cl}{\underset{\mid}{N}}CH_2CH_2OH$	272	10^{-2} M—no inhibition	
Triethylacetyl choline iodide	 $(C_2H_5)_3\underset{I}{\underset{\mid}{N}}CH_2CH_2O\underset{O}{\underset{\|}{C}}CH_3$	315	10^{-2} M—no inhibition	

However, the average value showing that 14,000 times as many molecules of choline are required than of Ach to produce a given effect, is probably a close approximation to the relative activities of these two compounds on the *Venus* heart. Thus the acetic acid ester of choline is far more active than is the parent compound. This is an observation that has been made by many workers on many tissues and organs.

The several esters of choline which were tested and the one ether (ethoxycholine) were all far more active than choline, with the exception of benzoylcholine which has approximately the same level of activity. The presence of the ring structure at the non-polar end of the molecule obviously affects the activity greatly. It is of interest to note that the substitution of chlorine for a hydrogen atom of the terminal methyl group in Ach to yield chloracetylcholine reduces the activity approximately one thousand fold.

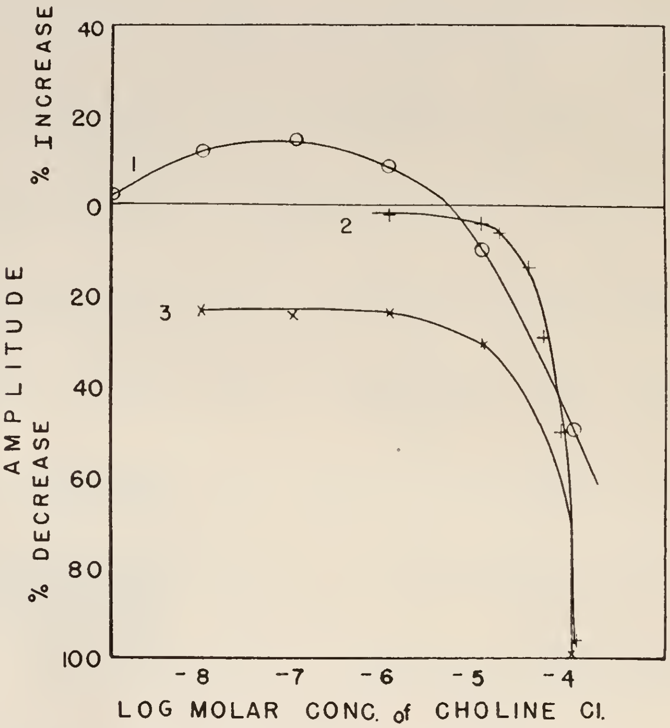


FIGURE 2. Showing the extreme variation in response of three different isolated *Venus* hearts to choline. Heart number 1 responded by an increase in amplitude to low concentrations of choline while hearts 2 and 3 showed only a decrease in amplitude, but their sensitivities differed markedly.

Betaine, a naturally occurring compound closely related to choline, is approximately equivalent to choline in its ability to depress the beat of the *Venus* heart, while the ethyl ester of betaine was found to be considerably more active than betaine.

A clear indication of the importance of the methyl groups attached to the nitrogen was seen when triethylcholine and triethylacetylcholine were tested. In the highest concentrations employed (10^{-2} M), neither of these produced the slightest degree of inhibition of heart beat. We have observed a similar striking difference in the actions of the tetramethyl ammonium ion, which decreases the amplitude of beat of the *Venus* heart, and tetraethyl-, tetra-n-propyl-, and triethyl-n-octyl ammonium ions, all three having an excitatory action only. These results obtained with the quaternary ammonium ions will be reported more extensively in a separate paper.

Importance of the methyl groups attached to the onium element directs attention to this portion of the Ach molecule. It is apparent from the present study and from similar earlier studies that different choline esters differ in their degree of pharmacological activity. This is also true of the *Venus* heart, but they all produce a characteristic decrease in amplitude. The substitution of ethyl for methyl groups in choline and Ach yields molecules which are completely lacking in inhibitory activity when applied to the isolated *Venus* heart. It has been indicated elsewhere (Welsh, 1948) that this specificity of the $(CH_3)_3N$ group, the rapidity of action of and recovery from Ach, and its activity in small amounts, suggest that Ach acts at the surface of cells as a "trigger" to set off a reaction or chain of reactions in the manner of an unstable coenzyme. Thus, the condition of the cell membrane is altered and the cell excited and then depressed depending on the concentration and time of action of the Ach. In further testing of this hypothesis, it is believed that the isolated *Venus* heart will continue to provide an ideal experimental object.

SUMMARY

1. A method of preparing and employing the isolated heart of the quahog, *Venus mercenaria,* for the bio-assay of acetylcholine (Ach) is described.

2. The activities of choline and certain choline esters; of betaine and its ethyl ester; and of triethylcholine and triethyl-acetylcholine on the isolated *Venus* heart are compared. In further understanding the fundamental mode of action of Ach, the most significant observation was that the substitution of ethyl groups for methyl on the nitrogen of choline and Ach resulted in a complete loss of activity determined by observation on the amplitude of heart beat.

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INCIDENCE AND ORIGIN OF ANDROGENETIC MALES IN X-RAYED HABROBRACON EGGS[1]

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INTRODUCTION

The terms androgenesis and merogony are sometimes used interchangeably. The former is defined by Wilson (1925) as "the activation of the egg by the sperm followed by development without the participation of the egg nucleus;" the latter as the "development of an egg fragment devoid of a nucleus fertilized by a normal sperm." This distinction is kept in this paper.

The study was undertaken with two objects in view: first, to determine whether low incidence of androgenetic males or high embryonic mortality is responsible for the low ratio of their occurrence as adults; and second, to work out the cytological mechanism underlying androgenesis in *Habrobracon*.

The literature dealing with androgenesis is considerable. Much of it is summarized in Wilson (1925), Sharpe (1934) and Darlington (1937). Three papers have been selected for discussion here. Packard (1918) exposed unfertilized *Chaetopterus* eggs in first meiotic metaphase to radium, and fertilized them with untreated sperm. When exposure was relatively long (35 to 50 minutes) the egg nucleus remained attached to the second polar body, the sperm nucleus divided, and development was androgenetic. Hasimoto (1934) identified some silkworm (*Bombyx*) males as androgenetic. These had developed from eggs which had been exposed to high temperature at time of oviposition when the eggs were undergoing the maturation divisions. These males were diploid, and by means of appropriate genetic combinations, he was able to demonstrate that they arose from the "union of two sperm nuclei in the egg cytoplasm without fertilization with the egg nucleus." Polyspermy is the rule in *Bombyx*. The third paper (Astaurow, 1937) describes the production of androgenetic males, likewise in *Bombyx*. They were produced along with the expected classes either by thermo-activation (40° C. for one hour) after fertilization, or by irradiation of the egg followed by fertilization with untreated sperm, or by both. At X-ray doses lethal to the egg nucleus, androgenetic males developed only after thermo-activation of the fertilized eggs. Heat treatment is used in this form to break diapause.

MATERIAL AND METHODS

For X-ray treatments a dual-tube self-rectifying outfit with a simultaneous cross-firing technique was used. The secondary voltage was 182 kv. and the tube cur-

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[1] This investigation was completed with the aid of a research grant from the National Cancer Institute of the National Institute of Health, U. S. Public Health Service. The author is also grateful to the University of Pennsylvania and to the Marine Biological Laboratory, Woods Hole, Massachusetts, for use of laboratory facilities, and to Mr. L. R. Hyde for administering the X-ray treatments. The drawings were made by Mrs. Jean Wilson.

rent on each tube was 25 ma. The heavy glass of the tube walls and 5 mm. of bakelite of the tube shields gave the filtering value of 0.2 mm. copper shield. The output intensity was 7210 r per minute, distance 9.5 cm. All breeding was carried on at 30° C.

Well-fed wild type females of the parasitic wasp *Habrobracon juglandis* were X-rayed and mated to untreated males which differed from wild type by one or more recessive traits. Eggs laid by these females during the first six hours after treatment had been X-rayed in late metaphase of the first meiotic division (metaphase I); the majority of those laid after this time had been treated in first meiotic prophase (prophase I) (Whiting 1938). Lethal dose [2] for the former is about 2400 r; for the latter, about 54,000 r (Whiting, 1941).

From all control crosses of the type used in this study, only diploid biparental females and haploid gynogenetic males are produced. Therefore, when wild type females are mated to males with traits recessive to wild type, daughters are wild type, heterozygous for the recessive traits, while sons have maternal wild type genes only. If this kind of cross is made after the females have been X-rayed, there appear occasionally males which show all the recessive paternal traits (Whiting, 1946a). These males are normal in appearance, fully fertile and transmit paternal traits only. They are, therefore, androgenetic. Their fertility is proof that they are haploid since diploid males which may arise from certain crosses in *Habrobracon* are always sterile or nearly so.

Repeated tests have shown that androgenetic males arise from eggs treated in metaphase I, and of 381 such eggs observed, six only, 1.57 per cent, developed into these exceptional males (dose 14,420 r–28,840 r). Although lethal dose for the nuclei of these eggs is 2400 r, androgenetic males have developed in eggs X-rayed with dose as high as 54,000 r (Whiting 1946b).

Eggs laid during the first six hours after treatment (14,420 r–36,050 r) were collected at one hour intervals, punctured, and fixed in Kahle's fixative. They were stained with the Feulgen technique and mounted whole in Canada balsam.

OBSERVATIONS

Speicher (1936) found that the most advanced eggs in *Habrobracon* egg sacs are in "early anaphase of the first maturation" (the author prefers to call this late metaphase I), and described normal oogenesis following oviposition. After the egg is laid, the maturation spindle passes into telophase I. The second division follows immediately. The four haploid groups of chromosomes (1a, 1b, 2a, 2b) lie in a row roughly perpendicular to the egg surface. During anaphase II, polar nuclei 1a and 2a remain stationary, 1b moves close to 2a, and 2b (functional nucleus) sinks deeper into the egg, a membrane forming as it moves. Nucleus 1a soon disintegrates, 1b and 2a unite and form a metaphase plate which divides and then disintegrates. Cleavage is of the usual insect type, with nuclei moving about until blastoderm formation, when cell membranes first appear.

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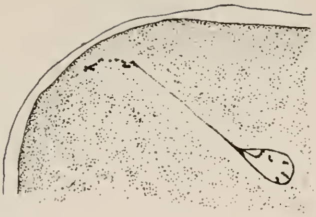
[2] Apparent inconsistencies in lethal doses in successive papers dealing with *Habrobracon* eggs are due to changes in method of calibration at the Marine Biological Laboratory. Conditions of treatment have not varied. In this paper all doses have been corrected for the latest measurements.

PLATE I



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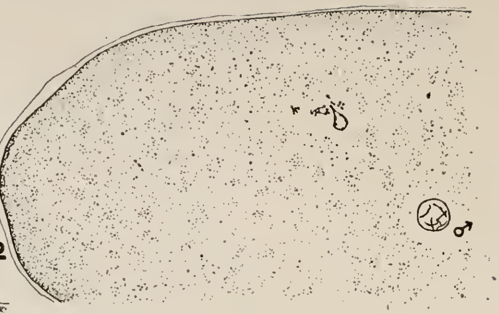

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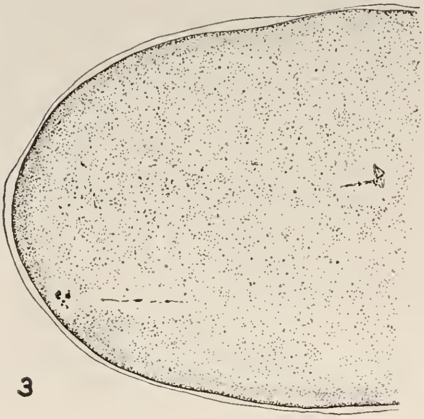

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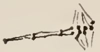

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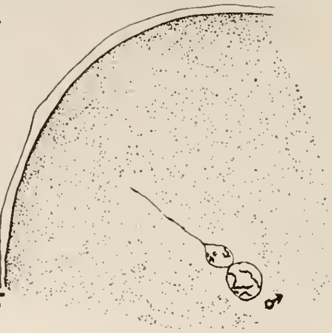

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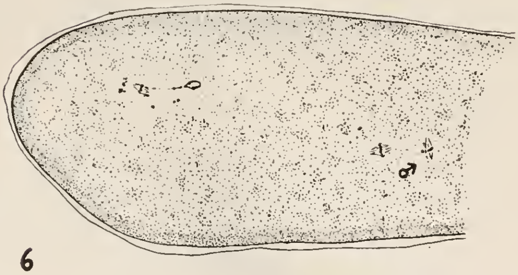

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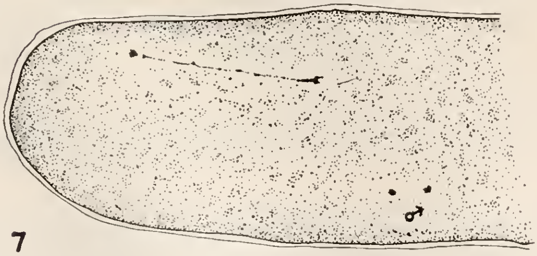

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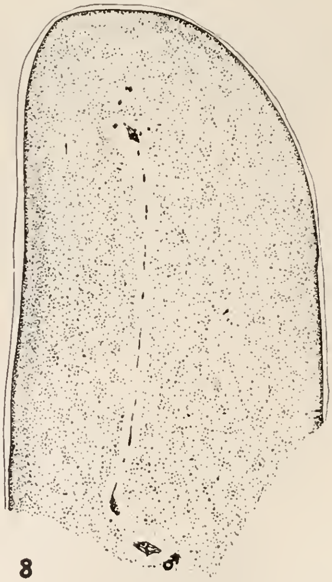

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After treatment with X-rays in prophase I, chromatin fragments, bridges, or both may occur in division I, or in division II, or in both divisions (Whiting, 1945a). Bridges occur but rarely in division II and when present appear to be single. After treatment in metaphase I, chromatin fragments (never bridges) may be seen between chromosome groups in telophase I and chromatin bridges appear in division II. These bridges may occur between nuclei 1a and 2a, or 1b and 2b, or between both pairs, never between 2a and 1b. They are made up of several chromatin threads and are present in both regions when dose is high. The attached egg pronucleus can move a long distance without breaking the bridges, and under these conditions it is pulled out into a "tear-drop" (Whiting, 1945b). Controls, of course, do not show fragments or bridges.

These bridges may retard the female pronucleus, but that they do not often stop it completely at relatively low doses is demonstrated by the fact that after lethal dose (2400 r) all but 2.4 per cent of unfertilized eggs develop well beyond first cleavage before death, and that these 2.4 per cent advance to first cleavage before dying (Whiting, 1945a).

These facts suggested to the author that the chromatin bridges formed during the second meiotic division of eggs X-rayed in metaphase I might sometimes retard the attached egg pronucleus to such a degree that the untreated sperm pronucleus would cleave before the egg pronucleus could reach it.

There were 702 eggs prepared and studied. Of these, 58.55 per cent were useless, either because they had been fixed at stages previous to syngamy or cleavage, or were not clearly stained; 41.45 per cent were of significance. They included all eggs undergoing syngamy as well as those in cleavage, where either chromosome number or presence or absence of chromosome aberrations demonstrated which pronuclei had taken part in cleavage. Of the 291 eggs fulfilling these requirements, three (1.03 per cent) only were found in which androgenetic development had begun (Plate 1, Figs. 6, 7 and 8). Three eggs not included in the 291 suggested incipient androgenesis. In each the male pronucleus was preparing for first cleavage while the female pronucleus was greatly retarded (Plate 1, Fig. 2). If these are accepted as androgenetic, a maximum of six among 294, or 2.04 per cent, is obtained. This does not differ significantly from 1.57 per cent of adult survivors, and demonstrates

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PLATE I

All illustrations were drawn with aid of a camera lucida from whole mounts of eggs which had been X-rayed in first meiotic metaphase. Fertilization was accomplished by untreated sperm. Anterior end and lateral view of each egg is shown. Cytoplasm is somewhat conventionalized. The lenses employed were a Spencer 2 mm. n. a. 1.3 apochromatic oil immersion objective and a × 5 or × 10 compensating ocular.

1. Attached pronucleus of unfertilized egg. Dose 28,840 r. × 650.
2. Attached and greatly retarded female pronucleus with normal male pronucleus. Egg X-rayed with 28,840 r. × 650.
3. First cleavage spindle of unfertilized egg. Chromatin bridges still attached to some cleavage chromosomes. Dose 14,420 r. × 370.
4. Cleavage spindle of Figure 3 in detail. × 650.
5. Syngamy. Egg X-rayed with 36,050 r. × 650.
6. Second cleavage of androgenetic development. Egg pronucleus greatly retarded. Egg X-rayed with 36,050 r. × 370.
7. Second cleavage of androgenetic development. Egg X-rayed with 28,840 r. × 370.
8. First cleavage of androgenetic development. Egg X-rayed with 14,420 r. × 370.

that there is little or no death of androgenetic embryos in spite of their development in cytoplasm irradiated with doses from six to fifteen times that lethal for the egg nucleus.

A study of Plate I will illustrate some points of cytology. It should be recalled here that *Habrobracon* chromosomes are extremely small. This will explain why they are not always represented exactly as to form and number ($n = 10$). In Plate I, Figure 1, is shown a typical "tear-drop" pronucleus. Polar nuclei are degenerating and suggest by their condition that the egg pronucleus is considerably retarded. Chromatin at the outer end of, as well as within, the egg pronucleus, is attached to bridges. This egg was not fertilized. Figures 3 and 4 show what can happen to such a tear-drop as that just described. The spindle is that of the first cleavage, and bridges with chromatin thickenings can be seen, still attached to chromosomes on the spindle. This is the only egg studied in which the chromatin connections could not be followed continuously from polar nuclei to egg pronucleus. What happens to a tear-drop pronucleus in the majority of cases when an X-rayed egg is fertilized after treatment, is shown in Figure 5. It unites with the normal male pronucleus and, in doing so, ultimately kills the embryo because of upset in chromosome balance due to chromatin loss.

Figure 2 strongly suggests an incipient androgenetic male. The egg pronucleus is so retarded and the male pronucleus so advanced that subsequent syngamy seems highly improbable.

Figures 6, 7, and 8 represent the only eggs found in which androgenetic development had begun. The isolation of the egg pronucleus from cleavage figures, with no evidence of any chromatin connection or remains between them and the normal appearance of the cleavage chromosomes, are to be noted. Figure 8 is especially convincing.

These three eggs have one thing in common which is rare in control eggs and not the rule in X-rayed ones: cleavage is taking place more posteriorly than one would expect. This suggests that some cytoplasmic factor, perhaps greater fluidity of the cytoplasm, may alter action of the sperm pronucleus so that it has moved "beyond the reach" of the impeded egg pronucleus.

Discussion

It is definitely established that androgenetic development (haploid) occurs in *Chaetopterus* and in *Habrobracon* after irradiation in metaphase I. Concerning *Bombyx*, Kawaguchi (1928) states, "Die Kerne in den Ovarialeiern der Schmetterlinge nach ihrem Ausschlüpfen aus der Puppe stehen fast immer im Stadium der Metaphase der ersten Reifteilung." This indicates that in *Bombyx* also treatment was given in metaphase I, since adult females were irradiated and then mated to untreated males.

That there is some special cytological response of tetrads to irradiation which causes chromatin bridges to be formed in division II, is apparent in both *Chaetopterus* and *Habrobracon*. The author (1945b) has discussed this in some detail but has been prevented from checking the theory completely by the small size of *Habrobracon* chromosomes. *Chaetopterus* chromosomes are relatively large and distinctive in character and should be analyzed in detail from this viewpoint. Packard describes chromatin bridges (dicentrics) in cleavage in eggs irradiated with doses low enough to permit the egg pronucleus to function in syngamy, a fact consistent with the conditions found in *Habrobracon.*

No cytological study of *Bombyx* eggs after treatment has been made. Hasimoto's conclusion, derived from genetic data, that androgenetic males (diploid) arise from the union of two sperm pronuclei, is not inconsistent with the suggestion that, in this form also, chromatin bridges retard the egg pronucleus.

That some viscosity change may be involved in *Habrobracon* androgenesis as well as chromatin attachment of the egg pronucleus to the polar nucleus, has been mentioned above. Hasimoto and Astaurow obtained androgenetic males in *Bombyx* by thermo-activation alone during meiotic divisions and after fertilization, and this may be evidence for viscosity change and movement of sperm pronuclei from their usual position. However, in *Chaetopterus* the sperm chromosomes remain in their normal position, close to those of the egg, yet syngamy does not occur.

A comparison of other factors in the two insect genera shows further similarities. In both, the percentage of eggs developing into androgenetic adults is low—1.57 per cent in *Habrobracon;* never higher than 0.273 per cent in *Bombyx* (Astaurow, 1937). In the former, 54,000 r is the highest dose at which androgenetic males were obtained; in the latter, 27,000 r was the highest dose tested, and they were produced after this treatment. In the *Chaetopterus* study, treatments were measured in minutes of exposure to radium bromide. After 50 minutes' exposure, about 70 per cent of treated eggs underwent haploid cleavage. At longer treatments the percentage dropped, until prolonged exposure stopped cleavage altogether.

In *Chaetopterus,* incidence of androgenesis but not adult survival; in *Bombyx,* adult survival but not incidence; and in *Habrobracon,* both incidence and adult survival, have been determined.

SUMMARY

1. 1.57 per cent of *Habrobracon* eggs X-rayed in first meiotic metaphase (14,420 r–28,840 r) and laid by treated females mated to untreated males develop into androgenetic males. These will develop after any dose up to 54,000 r although lethal dose for the egg chromosomes in this stage is 2400 r. Cytological study of 294 such eggs (14,420 r–36,050 r) shows that three were undergoing androgenetic cleavage while three others were possibly preparing for it. A maximum of six, or 2.04 per cent, does not differ significantly from androgenetic survivors, and it must be concluded that androgenetic embryos at doses used are as viable as embryos developing in untreated eggs.

2. Chromatin bridges which appear in meiotic division II after treatment in first meiotic metaphase retard and distort the egg pronucleus, occasionally to such a degree that the sperm pronucleus cleaves and develops into a normal fertile haploid male with paternal traits only. The almost complete absence of these bridges after treatment in first meiotic prophase will explain the failure of androgenetic males to develop in these eggs.

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ERRATUM

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The relation of the plasma membrane, vitelline membrane and jelly in the egg of Nereis limbata. Donald P. Costello.

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